WEST Search History

updated Search

DATE: Tuesday, September 02, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT; PLUR=YES; OP=AND			
L1	conjug$.clm.	17567	L1
L2	L1 and fibronectin$	252	L2
L3	L1 and (fibronectin$ and $fibronectin)	244	L3
L4	L1 and (fibronectin$ or $fibronectin)	252	L4
L5	L4 and (domain.clm. or edb.clm. or ed-b.clm. or eiiib.clm. or eiii-b.clm. or e-iii-b.clm. or elllb.clm. or elll-b.clm. or e-lll-b.clm. or e111b.clm. or e-111b.clm. or e-111-b.clm. or e111-b.clm.)	22	L5
L6	(edb.clm. or ed-b.clm. or eiiib.clm. or eiii-b.clm. or e-iii-b.clm. or elllb.clm. or elll-b.clm. or e-lll-b.clm. or e111b.clm. or e-111b.clm. or e-111-b.clm. or e111-b.clm.)	5	L6
L7	antiedb.clm.	0	L7
L8	anti-edb.clm.	0	L8
L9	edbconjugate.clm.	0	L9

END OF SEARCH HISTORY

WEST Search History

DATE: Tuesday, September 02, 2003

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=AND		
L1	radionuclide.clm.	903	L1
L2	L1 and (coagulation or co-agulat$ or occlusion or occlud$).clm.	7	L2
L3	L1 and tin.clm.	19	L3
L4	L3 and antibod$	8	L4
L5	L4 and fibronect$	0	L5
L6	L4 and isoform	0	L6

END OF SEARCH HISTORY

9/03 lbf updated

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Search Results - Record(s) 1 through 1 of 1 returned.

☐ 1. Document ID: US 6533819 B1

L1: Entry 1 of 1 File: USPT Mar 18, 2003

DOCUMENT-IDENTIFIER: US 6533819 B1
TITLE: Injectable implants for tissue augmentation and restoration

CLAIMS:

8. The method of claim 4 wherein said second peptide unit comprises a cell attachment sequence selected from the group consisting of Type-III domains of fibronectin, vitronectin, tenascin, titin and other related cell attachment proteins.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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Terms	Documents
domain8.clm. or domain-8.clm. or ((type-III.clm. or typeIII.clm. or typeiii.clm. or type111.clm. or type-lll.clm.) same fibronectin$.clm.)	1

Display F rmat: KWIC | Change Format

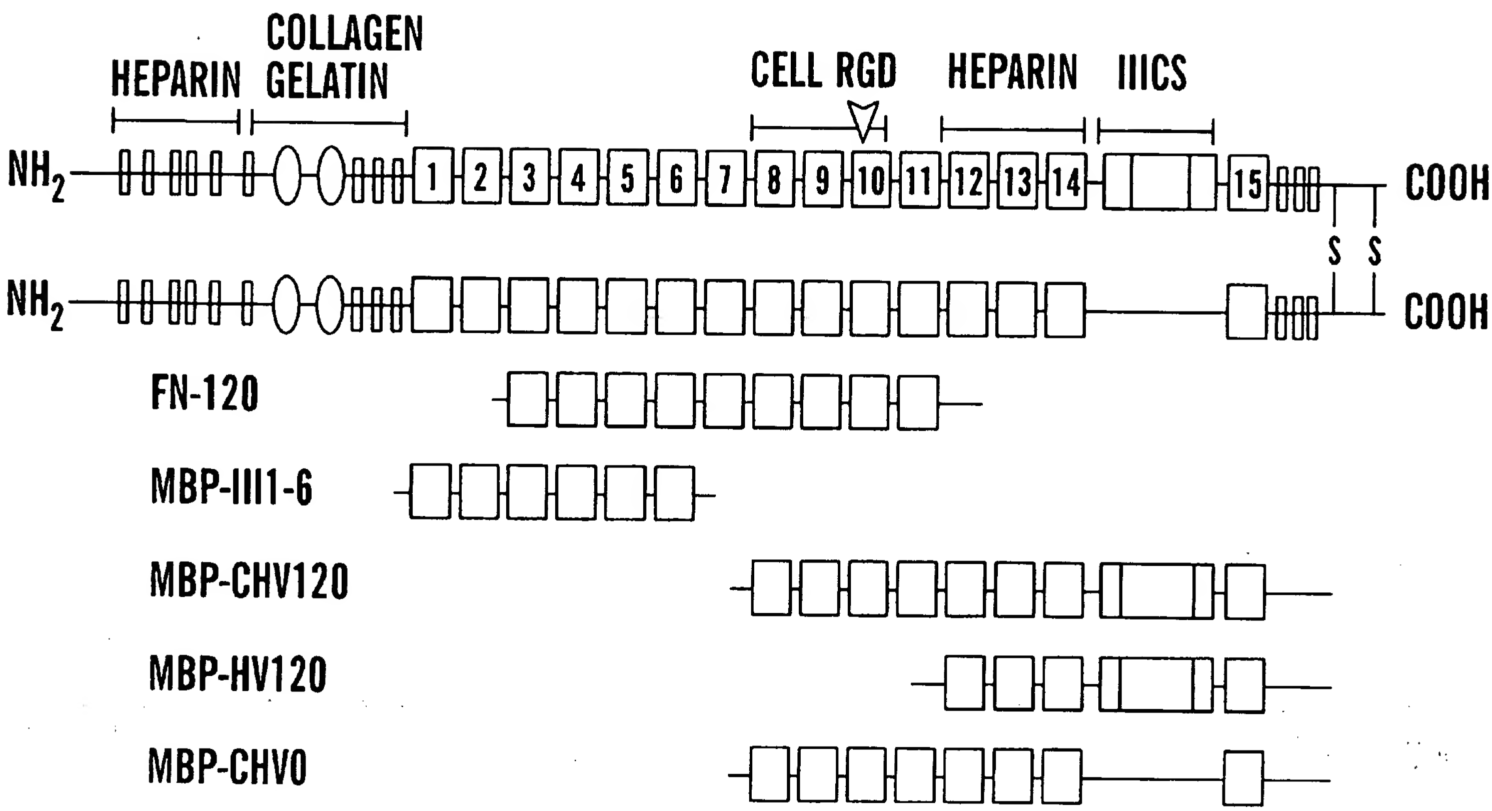
COLLAGEN
HEPARIN
GELATIN
CELL RGD
HEPARIN
IIICS
NH2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
COOH
S
S
NH2
COOH
FN-120
MBP-III1-6
MBP-CHV120
MBP-HV120
MBP-CHV0

FIG. 4

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antibodies, the IMR-32 cells also retracted their processes and many cells lifted off the dish yielding fewer cells after 2 days of incubation. No effect was observed with control antibodies. Thus, the neurite growth, differentiation and survival induced by the carbonic anhydrase-like domain of RPTP.beta. is mediated by contactin present in the neurons.

Other Reference Publication (9):
Brummendorf, T., M. J. Wolff, R. Frank, and F. G. Rathjen. 1989. Neural cell recognition molecule F11: homology with fibronectin type III and immunoglobulin type C domains. Neuron 2:1351-1361.

Other Reference Publication (11):
Brummendorf, T., and F. G. Rathjen. 1993. Axonal glycoproteins with immunoglobulin- and fibronectin type III-related domains in vertebrates: structural features, binding activities and signal transduction. Journal of Neurochemistry 61:1207-1219.

Other Reference Publication (52):
Norenberg, U., H. Willie, J. M. Wolff, R. Frank, and F. G. Rathjen. 1992. The chicken neural extracellular matrix molecule restrictrin: similarity with EGF-, fibronectin type III-, and fibrinogen-like motifs. Neuron 8:849-863.

CLAIMS:

1. A method for screening a test compound for the ability to alter the effects of the carbonic anhydrase domain of receptor-type phosphatase.beta. on neuronal cells, which comprises:

(a) growing neuronal cells expressing contactin in the presence of the carbonic anhydrase domain of receptor-type phosphatase.beta. and in the presence or absence of a test compound in solution, wherein in the absence of said test compound, said contactin and carbonic anhydrase domain bind;

(b) detecting a contactin-mediated characteristic of the neuronal cells, said characteristic selected from the group consisting of cell adhesion, outgrowth, differentiation, survival, and neurite extension; and

(c) comparing the characteristics detected in step (b) when the cells are exposed to the test compound with when the cells are not exposed to the test compound, wherein if the test compound enhances, mimics, or inhibits a contactin-mediated characteristic, then the test compound alters the effects of the carbonic anhydrase domain of receptor-type phosphatase.beta..

2. The method of claim 1 where the carbonic anhydrase domain of receptor-type phosphatase.beta. is coated on a solid substrate.

3. The method of claim 1 or claim 2 wherein the test compound is a peptide or protein, and the method further comprises:

(a) binding and covalently linking the test compound to the contactin to form a conjugate;

(b) affinity purifying the conjugate;

(c) cleaving the conjugate to reform the test compound and contactin; and

(d) determining the amino acid sequence of the test compound.

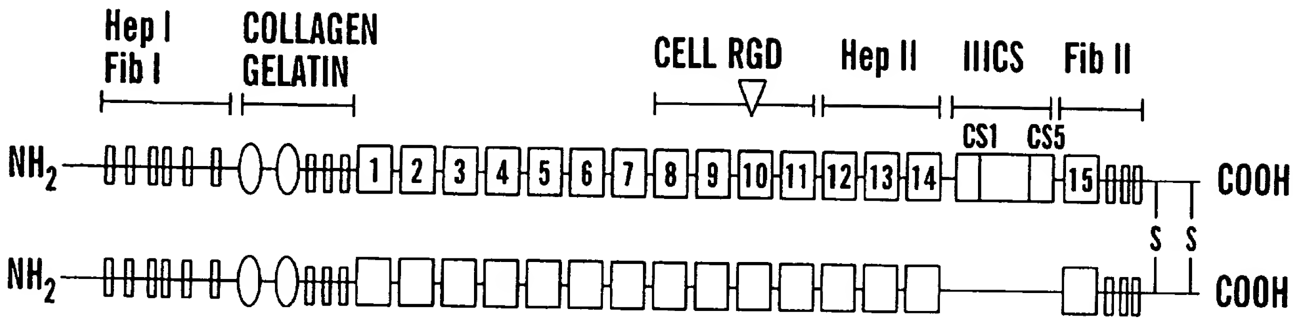

FIG. 2

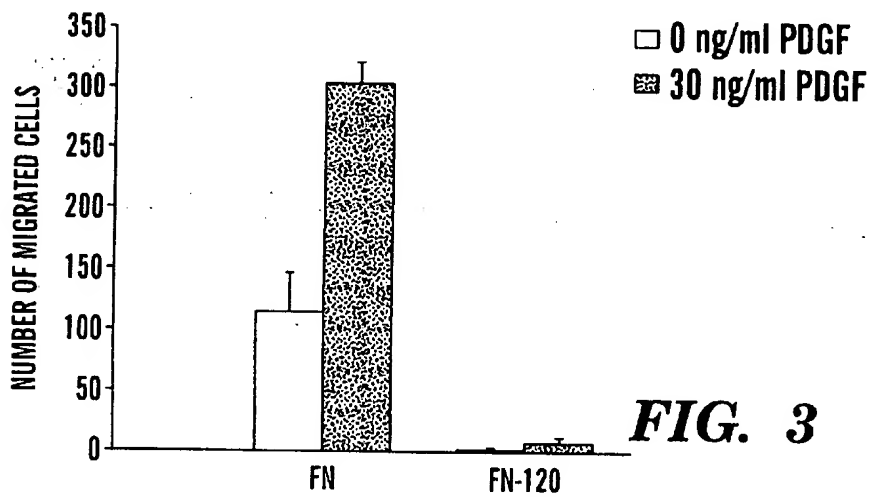

FIG. 3

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L5: Entry 8 of 22 File: USPT Jul 25, 2000

US-PAT-NO: 6093399
DOCUMENT-IDENTIFIER: US 6093399 A
** See image for Certificate of Correction **

TITLE: Methods and compositions for the specific coagulation of vasculature

DATE-ISSUED: July 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thorpe; Philip E.	Dallas	TX		
Edgington; Thomas S.	La Jolla	CA		

US-CL-CURRENT: 424/182.1; 424/178.1, 424/179.1, 424/180.1, 530/387.1, 530/387.3, 530/387.7, 530/387.9, 530/388.1, 530/388.22, 530/388.85, 530/391.7, 530/391.9

CLAIMS:

What is claimed is:

1. A binding ligand comprising:

(a) a first binding region that binds to a component expressed, accessible to binding or localized on the surface of a tumor cell, intratumoral vasculature or tumor stroma; the first binding region operatively linked to

(b) a coagulation factor or a second binding region that binds to a coagulation factor; wherein said second binding region comprises an antibody or an antigen binding region of an antibody.

2. The binding ligand of claim 1, wherein said first binding region comprises an IgG antibody or an IgM antibody.

3. The binding ligand of claim 1, wherein said first binding region comprises an antigen binding region of an antibody.

4. The binding ligand of claim 3, wherein said first binding region comprises an scFv, Fv, Fab', Fab or F(ab').sub.2 fragment of an antibody.

5. The binding ligand of claim 3, wherein said first binding region comprises an antigen binding region of an antibody that binds to a cell surface antigen of a tumor cell.

6. The binding ligand of claim 5, wherein said first binding region comprises an antigen binding region of an antibody that binds to the cell surface tumor antigen p185.sup.HER2, milk mucin core protein, TAG-72, Lewis a, carcinoembryonic antigen (CEA) or a tumor-associated antigen that binds to an antibody selected frin the group consisting of B3 (ATCC HB10573), KS1/4 (NRRL B/18536 and NRRL B/18537), 260F9 (ATCC HB 8488) and D612 (ATCC HB 9796).

7. The binding ligand of claim 3, wherein said first binding region comprises an antigen binding region of an antibody that binds to a component expressed, accessible to binding or localized on the surface of intratumoral vasculature.

8. The binding ligand of claim 7, wherein said first binding region comprises an antigen binding region of an antibody that binds to an intratumoral vasculature cell surface receptor.

9. The binding ligand of claim 8, wherein said first binding region comprises an antigen binding region of an antibody that binds to an MHC Class II protein, a VEGF/VPF receptor, an FGF receptor, a TGF.beta. receptor, a TIE, VCAM-1, P-selectin, E-selectin, .alpha..sub.v .beta..sub.3 integrin, pleiotropin, endosialin or endoglin.

10. The binding ligand of claim 9, wherein said first binding region comprises an antigen binding region of an antibody that binds to endoglin.

11. The binding ligand of claim 10, wherein said first binding region comprises an antigen binding region of an antibody that binds to the same epitope as the monoclonal antibody TEC-4 (ATCC HB-12312) or the monoclonal antibody TEC-11 (ATCC HB-12311).

12. The binding ligand of claim 9, wherein said first binding region comprises an antigen binding region of an antibody that binds to a VEGF/VPF receptor.

13. The binding ligand of claim 9, wherein said first binding region comprises an antigen binding region of an antibody that binds to VCAM-1.

14. The binding ligand of claim 9, wherein said first binding region comprises an antigen binding region of an antibody that binds to E-selectin.

15. The binding ligand of claim 9, wherein said first binding region comprises an antigen binding region of an antibody that binds to P-selectin.

16. The binding ligand of claim 7, wherein said first binding region comprises an antigen binding region of an antibody that binds to a ligand or growth factor that binds to an intratumoral vasculature cell surface receptor.

17. The binding ligand of claim 16, wherein said first binding region comprises an antigen binding region of an antibody that binds to FGF, TGF.beta., a ligand that binds to a TIE, a tumor-associated fibronectin isoform, scatter factor, hepatocyte growth factor (HGF), platelet factor 4 (PF4), PDGF or TIMP.

18. The binding ligand of claim 17, wherein said first binding region comprises an antigen binding region of an antibody that binds to FGF, TGF.beta., a ligand that binds to a TIE or a tumor-associated fibronectin isoform.

19. The binding ligand of claim 16, wherein said first binding region comprises an antigen binding region of an antibody that binds to the same epitope as the monoclonal antibody GV97 (ATCC HB-12451).

20. The binding ligand of claim 16, wherein said first binding region comprises an antigen binding region of an antibody that binds to the same epitope as the monoclonal antibody GV39 (ATCC HB-12450).

21. The binding ligand of claim 7, wherein said first binding region comprises an antigen binding region of an antibody that binds to an inducible intratumoral vasculature component.

22. The binding ligand of claim 21, wherein said first binding region comprises an antigen binding region of an antibody that binds to an intratumoral vasculature component inducible by a coagulant.

23. The binding ligand of claim 22, wherein said first binding region comprises an antigen binding region of an antibody that binds to an intratunoral vasculature component inducible by thrombin.

24. The binding ligand of claim 23, wherein said first binding region comprises an antigen binding region of an antibody that binds to P-selectin, E-selectin, PDGF or ICAM-1.

25. The binding ligand of claim 21, wherein said first binding region comprises an antigen binding region of an antibody that binds to an intratumoral vasculature component inducible by a cytokine.

26. The binding ligand of claim 25, wherein said first binding region comprises an antigen binding region of an antibody that binds to an intratumoral vasculature component inducible by a cytokine released by monocytes, macrophages, mast cells, helper T cells, CD8-positive T-cells or NK cells.

27. The binding ligand of claim 25, wherein said first binding region comprises an antigen binding region of an antibody that binds to an intratumoral vasculature component inducible by the cytokine IL-1, IL-4, TNF-.alpha., TNF-.beta. or IFN-.gamma..

28. The binding ligand of claim 25, wherein said first binding region comprises an antigen binding region of an antibody that binds to E-selectin, VCAM-1, ICAM-1, endoglin or an MHC Class II antigen.

29. The binding ligand of claim 28, wherein said first binding region comprises an antigen binding region of an antibody that binds to E-selectin.

30. The binding ligand of claim 28, wherein said first binding region comprises an antigen binding region of an antibody that binds to an MHC Class II antigen.

31. The binding ligand of claim 28, wherein said first binding region comprises an antigen binding region of an antibody that binds to VCAM-1.

32. The binding ligand of claim 7, wherein said first binding region comprises an antigen binding region of an antibody that binds to a ligand:receptor complex or a growth factor:receptor complex, but does not bind to the ligand or growth factor or to the receptor when the ligand or growth factor or the receptor is not in the ligand:receptor complex.

33. The binding ligand of claim 32, wherein said first binding region comprises an antigen binding region of an antibody that binds to the same epitope as the monoclonal antibody GV97 (ATCC HB-12451).

34. The binding ligand of claim 32, wherein said first binding region comprises an antigen binding region of an antibody that binds to the same epitope as the monoclonal antibody GV39 (ATCC HB-12450).

35. The binding ligand of claim 3, wherein said first binding region comprises an antigen binding region of an antibody that binds to a component of tumor stroma.

36. The binding ligand of claim 35, wherein said first binding region comprises an antigen binding region of an antibody that binds to tenascin.

37. The binding ligand of claim 35, wherein said first binding region comprises an antigen binding region of an antibody that binds to a basement membrane component.

38. The binding ligand of claim 35, wherein said first binding region comprises

an antigen binding region of an antibody that binds to an activated platelet.

39. The binding ligand of claim 35, wherein said first binding region comprises an antigen binding region of an antibody that binds to an inducible tumor stroma component.

40. The binding ligand of claim 39, wherein said first binding region comprises an antigen binding region of an antibody that binds to a tumor stroma component inducible by a coagulant.

41. The binding ligand of claim 40, wherein said first binding region comprises an antigen binding region of an antibody that binds to a tumor stroma component inducible by thrombin.

42. The binding ligand of claim 41, wherein said first binding region comprises an antigen binding region of an antibody that binds to RIBS.

43. The binding ligand of claim 1, wherein said first binding region comprises a ligand or receptor that binds to a component expressed, accessible to binding or localized on the surface of a tumor cell or intratumoral vasculature.

44. The binding ligand of claim 43, wherein said first binding region comprises a ligand that binds to a tumor cell surface receptor or a soluble binding domain of a receptor that binds to a ligand that binds to a tumor cell surface molecule.

45. The binding ligand of claim 43, wherein said first binding region comprises a ligand or receptor that binds to a component of intratumoral vasculature.

46. The binding ligand of claim 45, wherein said first binding region comprises a ligand that binds to an intratumoral vasculature endothelial cell surface receptor.

47. The binding ligand of claim 46, wherein said first binding region comprises VEGF/VPF, FGF, TGF.beta., a ligand that binds to a TIE, a tumor-associated fibronectin isoform, scatter factor/hepatocyte growth factor (HGF), platelet factor 4 (PF4), PDGF or TIMP.

48. The binding ligand of claim 47, wherein said first binding region comprises VEGF/VPF.

49. The binding ligand of claim 47, wherein said first binding region comprises FGF.

50. The binding ligand of claim 45, wherein said first binding region comprises a soluble binding domain of a cell surface receptor that binds to a ligand that binds to an intratumoral vasculature endothelial cell surface receptor.

51. The binding ligand of claim 50, wherein said first binding region comprises a soluble binding domain of a VEGF/VPF receptor.

52. The binding ligand of claim 1, wherein said first binding region is operatively linked to a coagulation factor.

53. The binding ligand of claim 52, wherein said first binding region is operatively linked to a Tissue Factor or a Tissue Factor derivative.

54. The binding ligand of claim 53, wherein said first binding region is operatively linked to a mutant Tissue Factor deficient in the ability to activate Factor VII.

55. The binding ligand of claim 54, wherein said first binding region is operatively linked to a Tissue Factor that includes a mutation in the amino acid region between about position 157 and about position 167.

56. The binding ligand of claim 55, wherein said first binding region is

operatively linked to a mutant Tissue Factor wherein Trp at position 158 is changed to Arg; wherein Ser at position 162 is changed to Ala; wherein Gly at position 164 is changed to Ala; or wherein Trp at position 158 is changed to Arg and Ser at position 162 is changed to Ala.

57. The binding ligand of claim 53, wherein said first binding region is operatively linked to truncated Tissue Factor.

58. The binding ligand of claim 57, wherein said first binding region is operatively linked to a truncated Tissue Factor that comprises amino acids 1 to about 219 of the mature Tissue Factor protein.

59. The binding ligand of claim 57, wherein said first binding region is operatively linked to a truncated Tissue Factor that has the amino acid sequence of SEQ ID NO:23.

60. The binding ligand of claim 53, wherein said first binding region is operatively linked to a first Tissue Factor or derivative operatively linked to at least a second Tissue Factor or derivative.

61. The binding ligand of claim 1, wherein said first binding region is operatively linked to a second binding region that binds to a coagulation factor, said second binding region comprising an antibody or an antigen binding region of an antibody.

62. The binding ligand of claim 61, further comprising a coagulation factor bound to said second binding region.

63. The binding ligand of claim 61, wherein said second binding region comprises an IgG antibody or an IgM antibody.

64. The binding ligand of claim 61, wherein said second binding region comprises an antigen binding region of an antibody that binds to a coagulation factor.

65. The binding ligand of claim 64, wherein said second binding region comprises an scFv, Fv, Fab', Fab or F(ab').sub.2 fragment of an antibody.

66. The binding ligand of claim 61, wherein said second binding region comprises an antibody or an antigen binding region of an antibody that binds to Tissue Factor or a Tissue Factor derivative.

67. The binding ligand of claim 66, wherein said second binding region comprises an antibody or an antigen binding region of an antibody that binds to a mutant Tissue Factor deficient in the ability to activate Factor VII.

68. The binding ligand of claim 67, wherein said second binding region comprises an antibody or an antigen binding region of an antibody that binds to a mutant Tissue Factor that includes a mutation in the amino acid region between about position 157 and about position 167.

69. The binding ligand of claim 68, wherein said second binding region comprises an antibody or an antigen binding region of an antibody that binds to a mutant Tissue Factor in which Trp at position 158 is changed to Arg; Ser at position 162 is changed to Ala; Gly at position 164 is changed to Ala; or wherein Trp at position 158 is changed to Arg and Ser at position 162 is changed to Ala.

70. The binding ligand of claim 66, wherein said second binding region comprises an antibody or an antigen binding region of an antibody that binds to truncated Tissue Factor.

71. The binding ligand of claim 70, wherein said second binding region comprises an antibody or an antigen binding region of an antibody that binds to a truncated Tissue Factor that comprises amino acids 1 to about 219 of the mature Tissue Factor protein.

72. The binding ligand of claim 70, wherein said second binding region comprises an antibody or an antigen binding region of an antibody that binds to a truncated Tissue Factor that has the amino acid sequence of SEQ ID NO:23.

73. The binding ligand of claim 66, wherein said second binding region comprises an antibody or an antigen binding region of an antibody that binds to a first Tissue Factor or derivative operatively linked to at least a second Tissue Factor or derivative.

74. The binding ligand of claim 1, wherein said binding ligand comprises the vitamin K-dependent coagulant Factor II/IIa, Factor VII/VIIa, Factor

IX/IXa or Factor X/Xa.

75. The binding ligand of claim 74, wherein said binding ligand comprises a vitamin K-dependent coagulation factor lacking the Gla modification.

76. The binding ligand of claim 75, wherein said vitamin K-dependent coagulation factor lacking the Gla modification is prepared by expressing a vitamin K-dependent coagulation factor-encoding gene in a procaryotic host cell.

77. The binding ligand of claim 75, wherein said vitamin K-dependent coagulation factor lacking the Gla modification is prepared by treating the vitamin K-dependent coagulation factor protein to remove or alter the corresponding Glutamic acid residues.

78. The binding ligand of claim 75, wherein said vitamin K-dependent coagulation factor lacking the Gla modification is prepared by preparing an engineered coagulation factor gene that encodes a vitamin K-dependent coagulation factor lacking the corresponding Glutamic acid residues and expressing said engineered gene in a recombinant host cell.

79. The binding ligand of claim 1, wherein said binding ligand comprises Russell's viper venom Factor X activator.

80. The binding ligand of claim 1, wherein said binding ligand comprises a platelet-activating compound.

81. The binding ligand of claim 80, wherein said binding ligand comprises thromboxane A.sub.2 or thromboxane A.sub.2 synthase.

82. The binding ligand of claim 1, wherein said binding ligand comprises an inhibitor of fibrinolysis.

83. The binding ligand of claim 82, wherein said binding ligand comprises .alpha.2-antiplasmin.

84. The binding ligand of claim 1, wherein said first binding region is operatively linked to said coagulation factor or said second binding region via a covalent bond or chemical cross-linker.

85. The binding ligand of claim 84, wherein said first binding region is operatively linked to said coagulation factor or said second binding region via a biologically releasable bond.

86. The binding ligand of claim 84, wherein said binding ligand is a fusion protein prepared by expressing a recombinant vector in a host cell, wherein the vector comprises, in the same reading frame, a DNA segment encoding said first binding region operatively linked to a DNA segment encoding said coagulation factor or second binding region.

87. The binding ligand of claim 1, wherein said first binding region is operatively linked to said coagulation factor or said second binding region using an avidin:biotin combination.

88. The binding ligand of claim 1, further defined as a bispecific antibody, wherein said first and second binding regions are antibodies or antigen binding regions of antibodies.

89. The binding ligand of claim 88, further defined as a bispecific antibody comprising a first antibody or antigen binding region that binds to an MHC Class II protein operatively linked to a second antibody or antigen binding region that binds to truncated Tissue Factor.

90. The binding ligand of claim 1, comprising an engineered coagulation factor.

91. The binding ligand of claim 1, dispersed in a pharmacologically acceptable carrier.

92. A binding ligand comprising a first binding region that binds to a component expressed, accessible to binding or localized on the surface of intratumoral vasculature or stroma, the first binding region operatively linked to a coagulant or to an antibody, or antigen binding region thereof, that binds to a coagulant.

93. A binding ligand comprising a first antibody, or antigen binding region thereof, that binds to a component expressed, accessible to binding or localized on the surface of intratumoral vasculature or stroma, wherein the first antibody or antigen binding region is operatively linked to a coagulant or to a second antibody, or antigen binding region thereof, th:at binds to a coagulant.

94. A binding ligand comprising a first antibody, or antigen binding region thereof, that binds to a marker expressed, accessible to binding or localized on the cell surface of intratumoral blood vessels of a vascularized tumor, wherein the first antibody or antigen binding region is linked to a coagulant or to a second antibody, or antigen binding region thereof, that binds to a coagulant.

95. A binding ligand comprising a first antibody, or antigen binding region thereof, that binds to a marker expressed or localized on the cell surface of intratumoral blood vessels of a vascularized tumor, wherein the first antibody or antigen binding region is linked to a coagulant or to a second antibody, or antigen binding region thereof, that binds to a coagulant.

96. A conjugate comprising a first antibody, or antigen binding portion thereof, that binds to a marker expressed or localized on the cell surface of intratumoral blood vessels of a vascularized tumor, wherein the first antibody or antigen binding portion is linked to a coagulant or to a second antibody, or antigen binding region thereof, that binds to a coagulant.

97. A binding ligand comprising a first antibody, or antigen binding region thereof, that binds to a marker expressed or localized on the cell surface of intratumoral blood vessels of a vascularized tumor, wherein the first antibody or antigen binding region is linked to a coagulant or to a second antibody, or antigen binding region thereof, that binds to a coagulant, said binding ligand being effective to promote coagulation in intratumoral blood vessels upon administration to an animal having a vascularized tumor.

98. The binding ligand of claim 97, wherein said first antibody, or antigen binding region thereof, is linked to a coagulant.

99. The binding ligand of claim 97, wherein said first antibody, or antigen binding region thereof, is linked to a second antibody, or antigen binding region thereof, that binds to a coagulant.

100. A binding ligand comprising a first binding region that binds to a component expressed, accessible to binding or localized on the surface of a tumor cell, established intratumoral vasculature or tumor stroma, the first binding region operatively linked to a coagulation factor or to an antibody, or antigen binding region thereof, that binds to a coagulation factor.

101. A binding ligand comprising a first binding region that binds to a component

expressed, accessible to binding or localized on the surface of a tumor cell, tumor-associated vasculature or tumor stroma, the first binding region operatively linked to a coagulation factor or to an antibody, or antigen binding region thereof, that binds to a coagulation factor.

102. A pharmaceutical composition comprising, in a pharmacologically acceptable form, a binding ligand that comprises:

(a) A first binding region that binds to a component expressed, accessible to binding or localized on the surface of a tumor cell, intratumoral vasculature or tumor stroma; the first binding region operatively linked to

(b) a coagulation factor or a second binding region that binds to a coagulation factor, wherein said second binding region comprises an antibody or an antigen binding region of an antibody.

103. The pharmaceutical composition of claim 102, formulated for parenteral administration.

11162203 98038287 PMID: 9370953

Gene expression and synthesis of fibronectin **isoforms in rat hepatic stellate cells. Comparison with liver parenchymal cells and skin fibroblasts.**

Xu G; Niki T; Virtanen I; Rogiers V; De Bleser P; Geerts A

Laboratory for Cell Biology and Histology, Free University of Brussels (V.U.B.), Belgium.

Journal of pathology (ENGLAND) Sep 1997, 183 (1) p90-8, ISSN 0022-3417 Journal Code: 0204634

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Fibronectins are multifunctional glycoproteins that are important components of the extracellular matrix in normal and fibrotic liver. Multiple **fibronectin** isoforms are generated from a single gene by alternative splicing of the primary transcript at the domains EIIIA, **EIIIB** , and V. The aim of this study was to investigate the **fibronectin** isoforms expressed by activated hepatic stellate cells, the most important connective tissue-producing cells in injured liver. Hepatocytes and skin fibroblasts were also studied for comparison. Activation of hepatic stellate cells in vivo was induced by injecting CCl4 twice weekly for 3 weeks. Activation in vitro was achieved by culturing cells on plastic. The level of activation was evaluated by alpha-smooth muscle actin immunocytochemistry. Steady-state levels of **fibronectin** isoform messenger RNA were examined by Northern hybridization analysis using specific cDNA probes for the EIIIA, **EIIIB** , and V domains. The de novo synthesis of **fibronectin** isoforms was examined by metabolic labelling and immunoprecipitation using domain-specific **monoclonal antibodies** . **Fibronectin** transcripts were not detectable in freshly isolated hepatic stellate cells from normal liver. Cultured hepatic stellate cells, as well as skin fibroblasts, expressed EIIIA+, **EIIIB** +, and V95+ transcripts. They were detectable as early as day 3 and increased with time in culture. At 3 days in culture, more than 90 per cent of stellate cells were alpha-smooth muscle actin-positive. In vivo activated hepatic stellate cells expressed EIIIA+ and V95+ transcripts; **EIIIB** + **fibronectin** mRNA was absent. Less than 20 per cent of in vivo activated stellate cells expressed alpha-smooth muscle actin. Freshly isolated parenchymal cells from normal liver as well as from CCl4-treated liver expressed V95+ transcripts, but were negative for EIIIA or **EIIIB** **fibronectin** mRNA. Immunoprecipitation results were in accordance with Northern hybridization analysis. Hepatic stellate cells in culture synthesized and secreted **fibronectin** molecules that contained EIIIA, **EIIIB** , and V fragments. Our results indicate that hepatic stellate cells synthesize and secrete **fibronectin** isoforms that are distinct from those of parenchymal cells.

11150279 98026246 PMID: 9378776

The alternatively spliced domains EIIIB **and EIIIA of human** fibronectin **affect cell adhesion and spreading.**

Hashimoto-Uoshima M; Yan Y Z; Schneider G; Aukhil I
Department of Periodontics, University of North Carolina, Chapel Hill, NC 27599-7450, USA.

Journal of cell science (ENGLAND) Sep 1997, 110 (Pt 18) p2271-80, ISSN 0021-9533 Journal Code: 0052457
Contract/Grant No.: DE-07801; DE; NIDCR
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

Fibronectin has a complex pattern of alternative splicing at the pre-mRNA level leading to the expression of different isoforms. The alternatively spliced domains **EIIIB** and EIIIA are known to be prominently expressed during development and wound healing. While the other spliced domain (CS-segment) is known to promote cell adhesion in a cell type specific manner, the biological functions of the spliced domains **EIIIB** and EIIIA are not well understood. In the present study, we have prepared expression proteins of specific domains of human **fibronectin** using a prokaryotic expression system and used the purified fragments to test their ability to support adhesion and spreading of cultured cells. Fragments from type-III domains #7 to #12 were prepared in various combinations to include or exclude the spliced domains **EIIIB** and EIIIA. The results indicate that cultured NIL fibroblasts adhere to many of the fragments tested. However, the cell adhesion and spreading are enhanced, especially at lower concentrations, to fragments including the domain **EIIIB** . The inclusion of domain EIIIA led to a decrease in the adhesion of cells and those that adhered did not spread well. When tested in a centrifugal cell adhesion assay, fragments including domain **EIIIB** resisted the detaching forces and stayed adhered. Fragments that included domain EIIIA were unable to resist the detaching centrifugal forces to the same extent as the fragments that included domain **EIIIB** alone. These results suggest that the spliced domain **EIIIB** may be serving important biological functions in enhancing cell adhesion and spreading. This is likely to be mediated by conformational effects because domain **EIIIB** alone neither exhibited any adhesive activity nor competed in inhibiting adhesion to fragments #7-10.

Tags: Human; Support, U.S. Gov't, P.H.S.

Descriptors: Alternative Splicing--physiology--PH; * **Fibronectins** --chemistry--CH; * **Fibronectins** --genetics--GE; Actins--analysis--AN; **Antibodies** , **Monoclonal** ; Cell Adhesion--physiology--PH; Cell Size --physiology--PH; Cloning, Molecular; Cytoskeleton--chemistry--CH; Cytoskeleton--immunology--IM; DNA Primers; Escherichia coli--genetics--GE; Extracellular Matrix--chemistry--CH; Extracellular Matrix--metabolism--ME; Fibroblasts--cytology--CY; Fibroblasts--physiology--PH; **Fibronectins** --metabolism--ME; Fluorescent **Antibody** Technique; Gene Expression Regulation, Bacterial--physiology--PH; Gravitation; Plastics; Protein Conformation; Protein Structure, Tertiary; Recombinant Proteins--immunology --IM

CAS Registry No.: 0 (Actins); 0 (Antibodies, Monoclonal); 0 (DNA Primers); 0 (Fibronectins); 0 (Plastics); 0 (Recombinant Proteins)

Record Date Created: 19971112
Record Date Completed: 19971112

1090756 97446192 PMID: 9299376

Increased expression of fibronectin **isoforms after myocardial infarction in rats.**

Ulrich M M; Janssen A M; Daemen M J; Rappaport L; Samuel J L; Contard F; Smits J F; Cleutjens J P

Cardiovascular Research Institute Maastricht, Department of Pathology, Universiteit Maastricht, Maastricht, The Netherlands.

Journal of molecular and cellular cardiology (ENGLAND) Sep 1997, 29 (9) p2533-43, ISSN 0022-2828 Journal Code: 0262322

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Fibronectin is a known chemoattractant for several cell types which play a role in the wound healing process, like fibroblasts, endothelial cells and macrophages. In addition, **fibronectin** generates a scaffold to which other extracellular matrix components can attach. The possible involvement of **fibronectin** in the wound healing process after myocardial infarction (MI) was investigated by studying the expression of **fibronectin** isoforms after induction of a MI in the rat. Deposition of plasma (pFN) and cellular **fibronectin** (cFN) protein was determined immunohistochemically, using **monoclonal antibodies** specific for cFN and polyclonal anti-human total FN (tFN **antibodies**). Expression of the mRNAs of total cFN and the embryonic isoforms EIIIA and **EIIIB** was investigated, using in situ hybridization (ISH). The ratio between EIIIA containing **fibronectin** (EIIIA+-FN) mRNA and total cFN mRNA was determined using a semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). cFN protein was present from day 4 until day 35 after infarction and was located around the area of infarction, in the epi- and endomyocardium and in the wall of larger vessels. pFN was found in the infarcted cardiomyocytes 1 day after the induction of the MI. From day 4 on pFN protein deposition was found in the border zone of the infarction and in the wall of larger vessels. pFN immunoreactivity remained present at high levels around the area of infarction and in the vessel wall throughout the entire period of investigation (90 days). From day 35 after the infarction pFN protein was detected in cardiomyocytes of the right ventricle and septum. cFN mRNA, determined by in situ hybridization, was present in the border zone of the infarcted area as early as 1 day after MI, and its expression peaked at 4 days after MI. Four days after MI the mRNA's coding for both the embryonic isoforms EIIIA and **EIIIB** could also be detected in the same area. Because expression of the EIIIA isoform was more abundant than the **EIIIB** isoform we only determined the percentage of the EIIIIA containing isoform from total FN. EIIIA+ mRNA was elevated 1 day after MI. We conclude that various **fibronectin** isoforms including the embryonic isoforms accumulate in the heart after MI. This suggests that these isoforms may play a role in the wound healing process in the left ventricle of the infarcted heart.

Tags: Animal; Male

Descriptors: **Fibronectins** --metabolism--ME; *Myocardial Infarction --metabolism--ME; **Fibronectins** --genetics--GE; Immunohistochemistry --methods--MT; In Situ Hybridization; Isomerism; Myocardium--metabolism--ME ; Myocardium--pathology--PA; Polymerase Chain Reaction--methods--MT; RNA, Messenger--metabolism--ME; Rats; Rats, Wistar

CAS Registry No.: 0 (Fibronectins); 0 (RNA, Messenger)

Record Date Created: 19971023

Record Date Completed: 19971023

10930657 97282965 PMID: 9137099

Expression of fibronectin **splicing variants in organ transplantation: a differential pattern between rat cardiac allografts and isografts.**

Coito A J; Brown L F; Peters J H; Kupiec-Weglinski J W; van de Water L
Department of Pathology, Beth Israel Hospital, Boston, Massachusetts, USA.

American journal of pathology (UNITED STATES) May 1997, 150 (5) p1757-72, ISSN 0002-9440 Journal Code: 0370502
Contract/Grant No.: GM-36812; GM; NIGMS; RO1 AI23847; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: AIM; INDEX MEDICUS

Allograft rejection is associated with infiltration of inflammatory cells and deposition of extracellular matrix proteins. The extent to which diversity in the extracellular matrix regulates inflammatory cell function in transplants remains unclear. One group of extracellular matrix proteins, termed **fibronectins** (FNs), exhibits inherent diversity as a consequence of alternative splicing in three segments: EIIIA, **EIIIB** , or V. Although the EIIIA segment has documented functions in mesenchymal cell differentiation, neither this segment nor the **EIIIB** segment have been tested for effects specific to leukocyte functions. By contrast, the V region can include the CS-1 segment to which leukocytes may adhere through alpha 4 beta 1 integrins. In this study, we demonstrate that EIIIA+, **EIIIB** +, and V+ FN variants are synthesized, primarily by macrophages in distinct temporal and spatial patterns in two rat cardiac transplant models: either with antigenic challenge, allografts, or without challenge, isografts. The ratio of EIIIA inclusion into FN increases by day 1 in allografts and isografts and remains high until allografts are rejected (approximately 7 days) but falls to normal levels in tolerated isografts (day 6). **EIIIB** + FN ratios in allografts peak later than do EIIIA+ FNs (day 4). **EIIIB** + FN ratios remain relatively low in isografts. Interestingly, EIIIA+ and **EIIIB** + FNs are deposited prominently in the myocardium of rejecting allografts in close association with infiltrating leukocytes, and FN expression and deposition are prominent at sites of infarction. By contrast, these FNs are largely restricted to the epicardium and to a lesser degree in the immediately adjacent myocardium in isografts. CS-1+ FNs increase in allografts and isografts at 3 hours after transplantation but are particularly prominent in allografts 1 to 3 days before rejection. Our data suggest that FN splicing variants have a differential role in the effector functions of leukocytes in allografts and isografts and provide a foundation for testing their function on leukocytes and a rationale for FN-based therapeutics to modulate allograft rejection in transplant recipients.

Tags: Animal; Comparative Study; Male; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: Alternative Splicing; * **Fibronectins** --genetics--GE; *Heart Transplantation--pathology--PA; Blotting, Northern; Cell Movement; **Fibronectins** --chemistry--CH; **Fibronectins** --metabolism--ME; Fluorescent **Antibody** Technique, Direct; Heart Transplantation--immunology--IM; In Situ Hybridization; Myocardium--chemistry--CH; Myocardium--metabolism--ME; Myocardium--pathology--PA; RNA Probes; RNA, Messenger--analysis--AN; Rats; Rats, Inbred BN; Rats, Inbred Lew; Ribonucleases; Staining and Labeling; Transplantation, Homologous; Transplantation, Isogeneic

CAS Registry No.: 0 (Fibronectins); 0 (RNA Probes); 0 (RNA, Messenger)

Enzyme No.: EC 3.1.- (Ribonucleases)

Record Date Created: 19970520

Record Date Completed: 19970520

10802574 97092082 PMID: 8937747

Fibronectin **isoform distribution in the mouse. II. Differential distribution of the alternatively spliced** EIIIB **, EIIIA, and V segments in the adult mouse.**

Peters J H; Chen G E; Hynes R O

Division of Pulmonary and Critical Care Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA.

Cell adhesion and communication (SWITZERLAND) Aug 1996, 4 (2) p127-48, ISSN 1061-5385 Journal Code: 9417027

Contract/Grant No.: P01CA17007; CA; NCI; P01HL4184; HL; NHLBI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The alternatively spliced **EIIIB** , EIIIA, and V segments of **fibronectin** (FN) show widespread codistribution in the mouse embryo, suggesting that **EIIIB** +, EIIIA+, and V+ isoforms serve to facilitate morphogenesis and organogenesis (Peters, JH, and Hynes, RO, 1996, this issue). To gain further clues to functions of these segments, we have used segment-specific anti-FN **antibodies** to perform immunofluorescence microscopy on tissue sections obtained from mice aged 9 to 15 weeks. Staining for each of the three spliced segments, relative to that for the total FN pool, was reduced in the adult as compared with the embryo. Anti-V **antibodies** produced patterns which were most similar to those obtained with anti-total FN **antibodies** , localizing to basement membranes, connective tissues subjacent to epithelia, walls of blood vessels, and cartilage. Anti-EIIIA **antibodies** produced the next most widespread pattern, which included prominent staining of the walls of blood vessels of all sizes, the lung interstitium, and smooth muscle associated with the gastrointestinal (GI), genitourinary (GU), and respiratory tracts. Although anti- **EIIIB** **antibodies** produced the faintest and most restricted pattern of staining, **EIIIB** + FN could be detected in the walls of some smaller blood vessels, smooth muscle of the GI, GU, and respiratory tracts, as well as within cartilaginous structures, and eye. There were quantitative and/or qualitative differences in the staining patterns produced by the three segment-specific **antibodies** in a variety of tissues, including liver, cartilage, synovium, cornea, muscle, peripheral nerve, and lymph node. These findings suggest that each of the spliced segments of the FN molecule may occupy unique physical or functional positions within the extracellular matrix of the adult mouse.

Tags: Animal; Comparative Study; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: **Fibronectins** --classification--CL; *Mice--metabolism--ME; *RNA Splicing; Extracellular Matrix--chemistry--CH; **Fibronectins** --analysis--AN; **Fibronectins** --genetics--GE; Fluorescent **Antibody** Technique, Indirect; Gene Expression Regulation; Mice --anatomy and histology--AH; Mice--embryology--EM; Organ Specificity

CAS Registry No.: 0 (Fibronectins)

Record Date Created: 19970314

Record Date Completed: 19970314

10802573 97092081 PMID: 8937746

Fibronectin **isoform distribution in the mouse. I. The alternatively spliced** EIIIB **, EIIIA, and V segments show widespread codistribution in the developing mouse embryo.**

Peters J H; Hynes R O

Division of Pulmonary Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA.

Cell adhesion and communication (SWITZERLAND) Aug 1996, 4 (2) p103-25, ISSN 1061-5385 Journal Code: 9417027

Contract/Grant No.: PO1CA17007; CA; NCI; PO1HL4184; HL; NHLBI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Fibronectins (FNs) are extracellular matrix glycoproteins that are essential for embryonic development. In order to gain clues to possible developmental roles played by the particular isoforms of FN, we used indirect immunofluorescence microscopy to examine and compare the distributions of the alternatively spliced **EIIIB** , EIIIA, and V segments, as well as the total pool of FNs, in serial sections from mouse embryos. **Antibodies** to each of these segments produced staining patterns that colocalized during gastrulation (E7.5) and during early morphogenesis of somites and notochord (E9.5). During the period of continuing organogenesis in the latter half of gestation (E10.5 to E16.5), the **antibodies** generally continued to produce similar staining patterns localized to epithelial basement membranes, stromal connective tissues, blood vessel walls, and muscles. However, as development proceeded, there was a gradual decline in the intensity of staining for the spliced segments relative to the total pool of FN, with a particularly noticeable decline in staining for **EIIIB** and EIIIA segments in certain glandular organs, including the liver. A specific reduction in expression of these latter two segments was also evident in the uterus and placenta at early timepoints in gestation. However, the most dramatic difference in the expression of the spliced segments occurred in developing hyaline cartilage, which showed a selective reduction in staining for the EIIIA segment that was evident in the axial skeletal precursors by E12.5 and complete throughout the embryo by E15.5. Our findings suggest that the alternatively spliced **EIIIB** , EIIIA, and V segments are included in the FN that is required for the morphogenesis of "FN dependent" structures, including somites, notochord, and the vasculature. Conversely, these segments would appear to play divergent, and sometimes exclusive, biological roles in specific tissues such as liver, cartilage, and placenta.

Tags: Animal; Comparative Study; Female; Pregnancy; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: Embryo--metabolism--ME; *Fetal Proteins--classification--CL; * **Fibronectins** --classification--CL; *Gene Expression Regulation, Developmental; *Mice--metabolism--ME; *RNA Splicing; Embryo--anatomy and histology--AH; Embryo and Fetal Development; Fetal Proteins--analysis--AN; Fetal Proteins--genetics--GE; **Fibronectins** --analysis--AN; **Fibronectins** --genetics--GE; Fluorescent **Antibody** Technique, Indirect; Gastrula --metabolism--ME; Gestational Age; Mice--embryology--EM; Muscle Proteins --analysis--AN; Myometrium--metabolism--ME; Nerve Tissue Proteins--analysis --AN; Organ Specificity; Placenta--metabolism--ME

CAS Registry No.: 0 (Fetal Proteins); 0 (Fibronectins); 0 (Muscle Proteins); 0 (Nerve Tissue Proteins)

Record Date Created: 19970314

Record Date Completed: 19970314

10677654 97026788 PMID: 8872966

Glomerular up-regulation of EIIIA and V120 fibronectin **isoforms in proliferative** immune **complex nephritis.**

Alonso J; Gomez-Chiarri M; Ortiz A; Seron D; Condom E; Lopez-Armada M J; Largo R; Barat A; Egido J

Division of Nephrology, Fundacion Jimenez Diaz, Universidad Autonoma, Madrid, Spain.

Kidney international (UNITED STATES) Sep 1996, 50 (3) p908-19, ISSN 0085-2538 Journal Code: 0323470

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Fibronectin : (FNs) comprise a family of adhesive glycoproteins that are prominent components of mesangial extracellular matrix and accumulate during glomerular injury. By alternative splicing of an unique mRNA precursor, various FN isoforms can be originated. In rat, three regions of the molecule are involved: EIIIA, **EIIIB** and V. Because specific FN isoforms are expressed in embryogenesis and wound healing, conditions characterized by cell migration and adhesion, we examined the pattern of FN isoforms in the mild and severe phases of a progressive **immune** complex proliferative nephritis in rats. We constructed specific probes to analyze the splicing pattern of FN pre-mRNAs by ribonuclease protection assays. FN mRNAs containing EIIIA, **EIIIB** and V regions increased along, the progression of nephritis, though the increment of **EIIIB** -FN mRNA was modest. However, different regulation of all these isoforms was observed. The percentage of FN mRNA containing the EIIIA exon versus total FN increased with the severity of the disease, while the percentage of FN mRNA containing the **EIIIB** exon decreased. Relative V-FN mRNA expression versus total FN mRNA increased only in the severe phase. By means of specific **antibodies** we also studied the presence of EIIIA, **EIIIB** and V-FN proteins in the kidney. In the normal glomerutus, EIIIA-FN protein was barely detectable in the mesangium, increasing in the mild phase of nephritis. In the severe phase of nephritis, increased EIIIA-FN was localized in the mesangium, in Bowman's capsule and in crescents. By contrast, **EIIIB** -FN protein in the glomerulus was absent even in the severe phase. V120-FN protein, an isoform that mediates the attachment of leukocytes through the VLA-4 integrin, was present in the mesangium and glomerular capillary loops in control animals, and increased in the severe phase of nephritis, coinciding with a strong leukocyte infiltration. In conclusion, our results show that during **immune** glomerular injury there were marked changes in the pattern of FN isoforms expression. Since those isoforms, particularly V120 isoform, are important in cell adhesion and migration, their up-regulation may facilitate the recruitment of cells into the injured glomeruli. The blockade of the interaction between V120-FN and infiltrating leukocytes may represent a new approach to the treatment of nephritis.

Tags: Animal; Female; Support, Non-U.S. Gov't

Descriptors: **Fibronectins** --chemistry--CH; * **Fibronectins** --genetics --GE; *Glomerulonephritis--metabolism--ME; *Kidney Glomerulus--metabolism --ME; Alternative Splicing--physiology--PH; Blotting, Northern; Disease Progression; **Fibronectins** --analysis--AN; Gene Expression--physiology--PH; Glomerulonephritis--immunology--IM; Immunohistochemistry; Isomerism; Kidney Glomerulus--chemistry--CH; Kidney Glomerulus--physiopathology--PP; Leukocytes, Mononuclear--immunology--IM; RNA Precursors--metabolism--ME; RNA Probes; Rats; Rats, Wistar; Ribonucleases; Up-Regulation--physiology

10209162 96010262 PMID: 7573372

Embryonic fibronectin **isoforms are synthesized in crescents in experimental autoimmune glomerulonephritis.**

Nickeleit V; Zagachin L; Nishikawa K; Peters J H; Hynes R O; Colvin R B

Department of Pathology, Massachusetts General Hospital, Boston 02114, USA.

American journal of pathology (UNITED STATES) Oct 1995, 147 (4) p965-78, ISSN 0002-9440 Journal Code: 0370502

Contract/Grant No.: DK-36807; DK; NIDDK; PO1HL41484; HL; NHLBI; R37CA20822; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: AIM; INDEX MEDICUS

Crescents are a severe and stereotyped glomerular response to injury that occur in several forms of glomerulonephritis that progress to renal failure. The key pathogenetic step that leads to glomerular scarring in unknown, but **fibronectin** (FN), the clotting system, macrophages, and proliferating parietal epithelial cells are known to participate. This study was designed to determine whether FN is synthesized locally, and in what molecular isoform, and whether cytokines known to promote FN synthesis are present in the crescent. Rats immunized with bovine glomerular basement membrane develop cellular crescents by 14 days and fibrous crescents and glomerulosclerosis by 35 days. In situ hybridization was performed with oligonucleotides specific for sequences common to all FN isoforms (total FN) or sequences specific for the alternatively spliced segments (EIIIA, **EIIIB** , and V). Throughout the time period (14, 21, and 35 days) all crescents and glomerular tufts contained cells with strong ISH signals for total and V+ mRNA, with the strongest signals present in large cellular crescents at day 21. In contrast, EIIIA+ and **EIIIB** + mRNAs showed maximal abundance within sclerosing crescents at 35 days. Protein deposition of EIIIA+, **EIIIB** +, and V+ FN isoforms was confirmed by immunofluorescence with segment-specific FN **antibodies** . Transforming growth factor-beta and interleukin-1 beta, both known to promote FN synthesis, were found in cellular crescents (days 14 and 21) and were still present, but greatly diminished, in the sclerotic phase (day 35). In summary, EIIIA-, **EIIIB** -, and V+ FN mRNA plasma isoforms predominate in cellular crescents, whereas in the fibrosing stage, mainly the oncofetal EIIIA+, **EIIIB** +, and V+ isoforms are synthesized and accumulate.

Tags: Animal; Support, U.S. Gov't, P.H.S.

Descriptors: Autoimmune Diseases--metabolism--ME; * **Fibronectins** --biosynthesis--BI; * **Fibronectins** --chemistry--CH; *Glomerulonephritis --metabolism--ME; Alternative Splicing; Autoimmune Diseases--pathology--PA; Base Sequence; Cattle; Embryo--metabolism--ME; **Fibronectins** --metabolism --ME; Fluorescent **Antibody** Technique, Indirect; Glomerulonephritis --pathology--PA; Immunoenzyme Techniques; In Situ Hybridization; Isomerism; Kidney Glomerulus--pathology--PA; Molecular Sequence Data; Oligonucleotide Probes--genetics--GE; RNA, Messenger--metabolism--ME; Rats; Rats, Inbred WKY; Staining and Labeling

CAS Registry No.: 0 (Fibronectins); 0 (Oligonucleotide Probes); 0 (RNA, Messenger)

Record Date Created: 19951109

Record Date Completed: 19951109

07567622 93022552 PMID: 1405493

Transforming growth factor-beta regulates increased ductus arteriosus endothelial glycosaminoglycan synthesis and a post-transcriptional mechanism controls increased smooth muscle fibronectin , **features associated with intimal proliferation.**

Boudreau N; Clausell N; Boyle J; Rabinovitch M

Department of Pathology, University of Toronto, Ontario, Canada.

Laboratory investigation; a journal of technical methods and pathology (UNITED STATES) Sep 1992, 67 (3) p350-9, ISSN 0023-6837

Journal Code: 0376617

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

BACKGROUND: In previous studies we established that there are developmentally regulated increases in endothelial hyaluronan (HA) and heparan sulfate (HS), and smooth muscle cell **fibronectin** (FN) related to the formation of intimal cushions, structures essential to the postnatal closure of the ductus arteriosus (DA). In this report, we investigated the mechanisms underlying these features to ascertain whether they were independently or coordinately regulated. EXPERIMENTAL DESIGN: We determined by assessing HA polymer size and by pulse labeling with [3H]glucosamine whether the increased glycosaminoglycans (GAGs) incorporated in DA compared with aorta (Ao) endothelial cell matrices reflected increased synthesis of HA and HS rather than decreased degradation. We assessed whether transforming growth factor-beta (TGF-beta) may be responsible for the increased DA endothelial GAGs and smooth muscle FN production by confirming the presence of TGF-beta in DA tissue using immunohistochemistry and by assessing the effect of adding neutralizing **antibodies** to the cell cultures. We next determined whether the level of regulation of the increase in FN in DA smooth muscle cells was transcriptional or post-transcriptional by relating protein synthesis to steady state mRNA levels and to mRNA levels after serum stimulation. Using northern blot analyses with specific probes, we also explored the possibility that the FN produced by the DA and Ao was qualitatively different in the proportion of isoforms containing the V95+ region associated with secretion or the **EIIIB** + region that has been related to migration. RESULTS: We observed that HA polymer size produced by DA and Ao endothelial cells was similar and we further verified using pulse labeling that the increase in DA compared with Ao endothelial GAGs reflected increased synthesis of HA and HS rather than decreased degradation. There was increased immunostaining for TGF-beta in DA compared with Ao tissue and we showed that TGF-beta neutralizing **antibodies** reduced synthesis of GAGs by the DA endothelial cells to the level of that seen in the Ao cells, but did not reduce DA smooth muscle cell FN synthesis. The increase in FN synthesis by DA compared to Ao smooth muscle cells was not associated with increased levels of steady-state mRNA for FN. Furthermore, following serum-stimulated increases in FN mRNA, the DA yielded greater amounts of FN protein compared to Ao smooth muscle cells. The increased FN production by the DA smooth muscle cells could not be attributed to a relative lack of degradation of FN protein or mRNA, or to qualitative differences which might influence secretion, as both cell types contained similar proportions of **EIIIB** + and V95+ isoforms of FN. CONCLUSIONS: These results would suggest that, in contrast to the TGF-beta dependent increase in DA endothelial GAG synthesis, the increase in DA smooth muscle FN synthesis arises through differences in post-transcriptional regulation that are likely independent of TGF-beta.

Tags: Animal; Support, Non-U.S. Gov't

Descriptors: Endothelium, Vascular--metabolism--ME; * **Fibronectins** --analysis--AN; *Glycosaminoglycans--metabolism--ME; *Muscle, Smooth, Vascular--chemistry--CH; *Protein Processing, Post-Translational; *Transforming Growth Factor beta--physiology--PH; Aorta; Blotting, Northern ; Cell Division; Cells, Cultured; Ductus Arteriosus; Endothelium, Vascular --cytology--CY; Endothelium, Vascular--ultrastructure--UL; **Fibronectins** --genetics--GE; **Fibronectins** --metabolism--ME; Glycosaminoglycans --genetics--GE; Heparitin Sulfate--genetics--GE; Heparitin Sulfate --metabolism--ME; Hyaluronic Acid--genetics--GE; Hyaluronic Ac

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11778085 99216534 PMID: 10196121

NMR structure of the human oncofoetal fibronectin ED-B domain, a specific marker for angiogenesis .

Fattorusso R ; Pellecchia M; Viti F; Neri P; Neri D; Wuthrich K

Institut fur Molekularbiologie und Biophysik, Eidgenossische Technische Hochschule Honggerberg, CH-8093 Zurich, Switzerland.

Structure with Folding & design (ENGLAND) Apr 15 **1999** , 7 (4) p381-90, ISSN 0969-2126 Journal Code: 100889329

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

BACKGROUND: The process of angiogenesis (i.e. the formation of new blood vessels from pre-existing ones) is fundamental to physiological processes such as reproduction, development and repair, as well as to pathological conditions such as tumor progression, rheumathoid arthritis and ocular disorders. The oncofoetal ED-B domain, a specific marker of angiogenesis, consists of 91 amino acid residues that are inserted by alternative splicing into the fibronectin (FN) molecule. RESULTS: The NMR structure of the ED-B domain is reported and reveals important differences from other FN type III domains. A comparison of the ED-B domain with the crystal structure of a four-domain FN fragment shows the novel features of ED-B to be located in loop regions that are buried at interdomain interfaces, and which therefore largely determine the global shape of the FN molecule. The negatively charged amino acids in this highly acidic protein are uniformly distributed over the molecular surface, with the sole exception of a solvent-exposed hydrophobic patch that represents a potential specific recognition site. Epitope mapping with 82 decapeptides that span the ED-B sequence revealed that three ED-B-specific monoclonal antibodies, which selectively target newly forming blood vessels in tumor-bearing mice, bind to adjacent regions on the ED-B surface. CONCLUSIONS: The NMR structure enables the identification of a large surface area of the ED-B domain that appears to be accessible in vivo, opening up new diagnostic and therapeutic opportunities. Furthermore, the mapping of specific monoclonal antibodies to the three-dimensional structure of the ED-B domain, and their use in angiogenesis inhibition experiments, provides a basis for further investigation of the role of the ED-B domain in the formation of new blood vessels.

Tags: Animal; Human; Support, Non-U.S. Gov't

Descriptors: *Antigens, Neoplasm--chemistry--CH; *Fibronectins--chemistry --CH; *Magnetic Resonance Spectroscopy; *Neovascularization, Pathologic --metabolism--ME; *Protein Conformation; *Protein Isoforms--chemistry--CH; *Protein Structure, Tertiary; Amino Acid Sequence; Antigens, Neoplasm --immunology--IM; Antigens, Neoplasm--physiology--PH; Binding Sites; Biological Markers; Epitopes--immunology--IM; Fibronectins--immunology--IM; Fibronectins--physiology--PH; Glycosylation; Mice; Mice, Nude; Models, Molecular; Molecular Sequence Data; Neoplasm Transplantation; Protein Isoforms--immunology--IM; Protein Isoforms--physiology--PH; Recombinant Fusion Proteins--chemistry--CH; Recombinant Fusion Proteins--immunology --IM; Recombinant Fusion Proteins--physiology--PH; Teratocarcinoma--blood supply--BS; Teratocarcinoma--metabolism--ME; Teratocarcinoma--pathology --PA

Molecular Sequence Databank No.: PDB/2FNB

CAS Registry No.: 0 (Antigens, Neoplasm); 0 (Biological Markers); 0 (Epitopes); 0 (Fibronectins); 0 (Protein Isoforms); 0 (Recombinant Fusion Proteins); 0 (fibronectin, glycosylated); 0 (oncofetal antigens)

Record Date Created: 19990610

Record Date Completed: 19990610

11148093 98024044 PMID: 9359110

Targeting by affinity-matured recombinant antibody **fragments of an angiogenesis associated fibronectin** isoform .

Neri D; Carnemolla B; Nissim A; Leprini A; Querze G; Balza E; Pini A; Tarli L; Halin C; Neri P; Zardi L; Winter G

Cambridge Centre for Protein Engineering-MRC Centre, UK.

Nature biotechnology (UNITED STATES) Nov 1997, 15 (1[illegible]) p1271-5, ISSN 1087-0156 Journal Code: 9604648

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The oncofetal fibronectin (B-FN) isoform is present in vessels of neoplastic tissues during angiogenesis but not in mature vessels. B-FN could therefore provide a target for diagnostic imaging and therapy of cancer. Phage display libraries have been used to isolate human antibody fragments with pan-species recognition of this isoform. We describe the use of these fragments in nude mice to target an aggressive tumor (grafted F9 murine teratocarcinoma). Imaging in real time was done by infrared photodetection of a chemically coupled fluorophore. The targeting was improved by use of affinity-matured fragments with low kinetic dissociation rates (koff = 1.5 x 10(-4) s-1) and also by engineering dimeric fragments via a C-terminal amphipathic helix.

Tags: Animal; Human; Support, Non-U.S. Gov't

Descriptors: *Fibronectins--diagnostic use--DU; *Immunoglobulin Fragments --diagnostic use--DU; *Neovascularization, Pathologic--diagnosis--DI; Amino Acid Sequence; Base Sequence; DNA Primers; Fibronectins--metabolism--ME; Immunoglobulin Fragments--metabolism--ME; Mice; Mice, Nude; Molecular Sequence Data; Recombinant Proteins--diagnostic use--DU; Recombinant Proteins--metabolism--ME; Teratocarcinoma--blood supply--BS; Teratocarcino ma--diagnosis--DI

CAS Registry No.: 0 (DNA Primers); 0 (Fibronectins); 0 (Immunoglobulin Fragments); 0 (Recombinant Proteins)

Record Date Created: 19971218

Record Date Completed: 19971218

04130404   Genuine Article#: RG069   Number of References: 1
Title: **THE FIBRONECTIN ISOFORM CONTAINING THE ED-B ONCOFETAL DOMAIN - A MARKER OF** ANGIOGENESIS **(VOL 59, PG 612, 1994)**
Author(s): **CASTELLANI P** ; VIALE G; DORCARATTO A; NICOLO G; KACZMAREK J; QUERZE G; ZARDI L
Journal: INTERNATIONAL JOURNAL OF CANCER, **1995** , V62, N1 (JUL 4), P118
ISSN: 0020-7136
Language: ENGLISH   Document Type: CORRECTION, ADDITION
Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences
Journal Subject Category: ONCOLOGY
Cited References:
    CASTELLANI P, 1994, V59, P612, INT J CANCER
?b 155
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            $4.27   0.231 DialUnits File34
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            $5.35 1 Types
    $9.62 Estimated cost File34
    $0.22 TELNET
    $9.84 Estimated cost this search
   $10.10 Estimated total session cost   0.312 DialUnits

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416,997 NO 9/2

From: Portner, Ginny
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Importance: High

10802574 97092082 PMID: 8937747
Fibronectin isoform distribution in the mouse. II. Differential distribution of the alternatively spliced EIIIB , EIIIA, and V segments in the adult mouse.
Peters J H; Chen G E; Hynes R O
Division of Pulmonary and Critical Care Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA.
Cell adhesion and communication (SWITZERLAND) Aug 1996, 4 (2) p127-48, ISSN 1061-5385 Journal Code: 9417027
Contract/Grant No.: P01CA17007; CA; NCI; P01HL4184; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

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Cell Adhesion and Communication, 1996, Vol. 4, pp. 127–148
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Fibronectin Isoform Distribution in the Mouse

II. Differential Distribution of the Alternatively Spliced EIIIB, EIIIA, and V Segments in the Adult Mouse

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(Received 19 January 1996; In final form 8 March 1996)

The alternatively spliced EIIIB, EIIIA, and V segments of fibronectin (FN) show widespread codistribution in the mouse embryo, suggesting that $EIIIB^+$, $EIIIA^+$, and V^+ isoforms serve to facilitate morphogenesis and organogenesis (Peters, JH, and Hynes, RO, 1996, this issue). To gain further clues to functions of these segments, we have used segment-specific anti-FN antibodies to perform immunofluorescence microscopy on tissue sections obtained from mice aged 9 to 15 weeks. Staining for each of the three spliced segments, relative to that for the total FN pool, was reduced in the adult as compared with the embryo. Anti-V antibodies produced patterns which were most similar to those obtained with anti-total FN antibodies, localizing to basement membranes, connective tissues subjacent to epithelia, walls of blood vessels, and cartilage. Anti-EIIIA antibodies produced the next most widespread pattern, which included prominent staining of the walls of blood vessels of all sizes; the lung interstitium, and smooth muscle associated with the gastrointestinal (GI), genitourinary (GU), and respiratory tracts. Although anti-EIIIB antibodies produced the faintest and most restricted pattern of staining, $EIIIB^+$ FN could be detected in the walls of some smaller blood vessels, smooth muscle of the GI, GU, and respiratory tracts, as well as within cartilaginous structures, and eye. There were quantitative and/or qualitative differences in the staining patterns produced by the three segment-specific antibodies in a variety of tissues, including liver, cartilage, synovium, cornea, muscle, peripheral nerve, and lymph node. These findings suggest that each of the spliced segments of the FN molecule may occupy unique physical or functional positions within the extracellular matrix of the adult mouse.

Keywords: Fibronectin, Alternative Spicing, Extracellular Matrix, Development, Cartilage, Blood Vessels, Basement Membranes

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INTRODUCTION

Fibronectins (FNs) comprise a family of large adhesive glycoproteins that differ from one another on the basis of alternative splicing at three sites (reviewed by Hynes, 1990; and Mosher, 1989). Two of the sites conform with type III structural repeats, and may be completely included or excluded. These are referred to as extra type III repeats A (EIIIA) and B (EIIIB) in the rat, whereas they have been called extra domains A (EDA, also referred to as ED1) and B (EDB, also referred to as ED2) in the human. The third site of splicing, which does not conform with any of the repeating modules of FN and is therefore called the variable or V region (IIICS in the human), is subject to total inclusion, partial inclusion, or total exclusion (reviewed by Schwarzbauer, 1991; ffrench-Constant, 1995).

FNs have been studied to a greater extent than other currently recognized adhesion proteins, possibly owing to their ready isolation from blood plasma via affinity chromatography on immobilized gelatin. Research on such "plasma FN" has produced major insights into the mechanisms of cell adhesion, migration, and cytoskeletal organization (Hynes, 1990; Mosher, 1989). For example, the arginine-glycine-aspartic acid (RGD) cell binding motif, now known to be shared by FN and several other adhesion proteins, was discovered during the course of studies to characterize the adhesive properties of plasma-derived FN and its proteolytic products (Pierschbacher and Ruoslahti, 1984). Plasma FN was also instrumental in the discovery and characterization of the integrin family of cell surface receptors for extracellular matrix (ECM) proteins (Hynes, 1990). It should be noted, however, that the bulk of plasma FN is the product of hepatocytes (Tamkun and Hynes, 1985), which omit the EIIIB and EIIIA segments and which include part or all of the V segment only about 50% of the time (Schwarzbauer, 1991). Although small quantities of EIIIA$^+$ and EIIIB$^+$ FNs are normally present in blood plasma, these isoforms nevertheless constitute a small fraction ($\sim$1% or less for each) of the total pool of plasma FN (Vartio et al., 1987; Peters et al., 1989; 1995). Therefore, nearly all of the *in vitro* research cited above was performed using plasma FN which is essentially $B^-A^-V^{\pm}$. Consistent with this composition, significant progress has been made on the function of the V region, which is known to contain binding sites for the α_4 class of integrins (Guan and Hynes; 1990, Mould et al., 1990). However, despite recent observations regarding potential roles for the EIIIA segment in cell adhesion and differentiation (Xia and Culp, 1994, 1995; Jarnigan et al., 1994), much less is known regarding the functions of the "extra" type III repeats.

In order to gain clues to the biological functions of the different alternatively spliced forms of FN, we have used segment-specific antibodies to determine the relative tissue distributions of the three spliced segments of FN in the mouse. In a companion article (Peters and Hynes, 1996, this issue), we have observed that the segments generally show widespread expression and colocalization during embryonic development. The most striking exception to this rule is developing hyaline cartilage, from which the EIIIA segment selectively disappears during the latter half of gestation. In this study, we have analyzed tissues from adult mice with similar staining methods. We find that there is reduced expression of each of the three spliced segments, relative to the total pool of FN, in the adult mouse as compared with the embryo. We also note wider tissue distribution patterns for the EIIIB and EIIIA segments than previously reported for human tissues (Carnemolla et al., 1989; Glukhova et al., 1989, 1990; Vartio et al., 1987). And, in comparisons of the staining patterns achieved with the different segment-specific antibodies, we detect several instances of qualitative and/or quantitative differences in distribution between the three segments. These findings offer further support for the contention that the alternatively spliced segments of FN constitute ECM components with tissue- and/or organ-specific functions.

METHODS

Preparation of Tissue Sections

Adult CD-1 mice (Charles River Labs, Wilmington, MA), varying in age from 8 weeks and 6 days to 15

weeks, were killed by cervical dislocation or by carbon dioxide inhalation. Tissues and organs were dissected free, washed in cold phosphate buffered saline (PBS) and, in most cases, fixed in 2.5% paraformaldehyde in PBS for 1 h on ice. Specimens were then incubated in cold 0.1 M glycine in PBS for 1 h, and then cold 0.6 M sucrose in PBS for 4–5 h. In certain cases (lung and eye), both unfixed and fixed specimens were analyzed, with similar findings (not shown). Lungs to be analyzed unfixed were gently instilled via the trachea with warm O.C.T. embedding medium (Baxter Scientific, McGaw Park, IL) diluted 1:1 with PBS, and then frozen over dry ice. All tissues, whether fixed or unfixed, were positioned in plastic molds containing O.C.T., frozen over dry ice, and stored at −70° C. In the case of bone marrow, the contents of a longitudinally cut femur were scraped directly into O.C.T. prior to freezing. A Frigocut Cryostat (Reichert-Jung) was used to cut serial 6–8 μm tissue sections onto Superfrost/plus slides (Fisher Scientific, Pittsburgh, PA), which were air-dried overnight at room temperature and then stored at −70° C pending immunostaining. In addition to immunofluorescence microscopic analysis (see below), representative sections from each tissue were also stained with hematoxylin and eosin.

Antibodies

Immunopurified rabbit antibodies to the V95 subsegment and to the EIIIB segment were prepared as described (Peters and Hynes, 1996, this issue). Briefly, the anti-V95 antibodies were raised to a V95 β-galactosidase fusion protein (Schwarzbauer et al., 1985) and purified on rat plasma FN Sepharose, whereas the anti-EIIIB antibodies were raised to an EIIIB-GST fusion protein and purified on EIIIB-maltose binding protein Sepharose (Peters et al., 1995). We have previously shown that mammalian EIIIB$^+$ FNs typically contain N-linked carbohydrate that causes interference with recognition of the EIIIB segment by antibodies. Therefore, target FNs, including those in tissue sections, generally require deglycosylation in order to permit recognition by such antibodies (Peters et al., 1995; Peters and Hynes, 1996, this issue). Except for the instances noted below, the anti-EIIIB antibody staining patterns reported in this study were only present in tissue sections that were pretreated with N-glycanase as described (Peters and Hynes, 1996, this issue).

Immunopurified goat antibodies (G153) to a 29 amino acid synthetic peptide of the human EDA (ED1) segment (Peters et al., 1988) were used to detect the EIIIA segment in adult mouse tissues. Because G153 was originally raised to and purified on human FN sequences, preliminary experiments were performed to test its capacity to recognize the EIIIA segment of rodent FNs. In Western blotting, G153 recognized EIIIA$^+$ but not EIIIA$^-$ full length recombinant rat FNs (Guan et al., 1990), and specifically stained only a minor high molecular weight subpopulation of subunits that resolved under reducing conditions from purified mouse plasma FN (Gibco-BRL) (Vartio et al., 1987; Peters et al., 1988; data not shown). In immunofluorescence microscopy of tissue sections obtained from mouse embryos (E7.5 through E16.5), G153 produced patterns of fibrillar staining which were similar to those reported for the monoclonal antibody 3E2 (Peters and Hynes, 1996, this issue).

To stain adult mouse tissue sections for the total pool of FNs ("total FN"), rabbit antisera to rat cellular FN (Mautner and Hynes, 1977; Peters and Hynes, 1996, this issue) was generally used. However, in certain instances immunopurified goat antibodies (G141), raised to and purified on rabbit plasma FN (Peters et al., 1986), were substituted. The staining patterns produced by G141 were indistinguishable from those produced by the rabbit anti-cellular FN antibodies (not shown).

Immunohistochemistry

To compare the tissue distributions of the three spliced segments of FN to one another, as well as to the total pool of FNs, we subjected adjacent or closely situated serial tissue sections from adult mice to indirect immunofluorescence staining with the anti-EIIIB, anti-EIIIA, anti-V, and anti-total FN antibodies. The method for detection of the EIIIA seg-

ment differed from that used in our analysis of the mouse embryo, in that G153 was used instead of monoclonal antibody 3E2. Also, G141 was occasionally substituted for the rabbit anti-total FN antibodies (see above). Otherwise, the staining methodology used to detect the EIIIB and V segments, as well as the total pool of FNs, was identical to that used in our analysis of embryos (Peters and Hynes, 1996, this issue). Anti-EIIIB, anti-EIIIA, and anti-V antibodies were used at final concentrations of 36–69, 50, and 4 μg/ml, respectively, unless indicated otherwise. Rabbit anti-total FN antiserum was used at a dilution of 1:1000, whereas immunopurified G141 was used at 2 μg/ml. In all cases, appropriate FITC-labeled secondary antibodies (Cappel, Durham, NC) were diluted 1:100 in 2% ovalbumin in PBS. In two instances in this study (Figures 5 and 6), we present comparisons of adult and embryonic staining patterns. In these instances, monoclonal antibody 3E2 was used to detect the EIIIA segment in the embryonic tissues, as previously described (Peters and Hynes, 1996, this issue).

The four criteria used to assure specificity of immunostaining in our analysis of embryonic mouse tissues (Peters and Hynes, 1996, this issue) were also employed in this study. Briefly, the following were assessed to be nonspecific: 1) staining patterns present in the absence of primary antibody, 2) segment-specific antibody patterns that were not "matched" by similar anti-total FN antibody patterns, 3) segment-specific patterns that were not "blocked" by an appropriate GST fusion protein. In certain instances, we also used the synthetic peptide to which G153 had been raised (Peters et al., 1988) to block staining and thereby assure specificity. Finally, enhancement of anti-EIIIB antibody staining patterns by N-glycanase, although not an absolute criterion for specificity (see below), was considered to be an attribute of specific antibody recognition if present (Peters et al., 1995; Peters and Hynes, 1996, this issue).

As was the case for the mouse embryo (Peters and Hynes, 1996, this issue), the anti-EIIIB antibodies used in this study produced N-glycanase-independent staining at the periphery of red blood cells (RBCs) in the adult mouse (Figure 8). In both embryos and adult mice, such staining appeared to be nonspecific, based upon the failure of anti-total FN antibodies (as well as the other segment-specific antibodies) to produce an equivalent pattern. Nonetheless, it was specifically blocked by coincubation of the antibodies with EIIIB-GST, but not irrelevant GST fusion proteins, suggesting recognition of a crossreactive epitope by the primary anti-EIIIB antibodies.

Fluorescence staining intensity was graded as follows: (−) negligible, (+) faint, (++) moderate, (+++) strong. These grades reflect the overall intensity of staining produced in a specific tissue, rather than in any particular structure within that tissue. Several major patterns of tissue staining were observed: (B) = basement membrane zone, (V) = capillary and small blood vessels, (P) = perimysial/endomysial or, in the case of peripheral nerve, perineurial/endoneurial, (S) = subepithelial connective tissue, (M) = mesothelial, (F) = hair follicle sheath.

RESULTS

Distribution of the Alternatively Spliced EIIIB, EIIIA, and V Segments of FN in the Adult Mouse. The staining patterns that were produced by the four different anti-FN antibodies in tissue sections from adult mice (Figures 1-8) are described below according to organ system. In addition, Table I summarizes the various staining patterns in tissues belonging to each organ system in quantitative and qualitative terms.

Cardiovascular System

Heart. None of the anti-FN antibodies produced appreciable staining of cardiac muscle fibers. However, anti-total FN, anti-EIIIA, and anti-V antibodies (in order of staining intensity) each produced staining of pericardium, endocardium, and myocardial interstitium. In the latter compartment, the staining produced by anti-total FN antibodies in capillary walls and within the endomysial fascia appeared to be of simi-

TABLE I Distribution of the Alternatively Spliced Segments of Fibronectin in the Adult Mouse*

ORGAN SYSTEM/TISSUE:	SEGMENT OR CATEGORY OF FIBRONECTIN TARGETED BY PRIMARY ANTIBODY			
	TOTAL FN	EIIIB	EIIIA	V95
CARDIOVASCULAR				
MYOCARDIUM	$+++^{V,P,M}$	−	$++^{V,P,M}$	$+^{V,P,M}$
VALVES	$+++^{S}$	−	$+^{S}$	$++^{S}$
AORTA	$+++^{P,S}$	−	$++^{P,S}$	$++^{P,S}$
MUSCULAR ARTERIES	$+++^{P,S}$	$+^{P}$	$++^{P,S}$	$++^{P,S}$
VEINS	$+++^{S}$	−	$++^{S}$	$++^{S}$
RESPIRATORY				
†TRACHEA	$+++^{B,V,P,S}$	−	$++^{B,V,P,S}$	$++^{B,V,P,S}$
†BRONCHI	$+++^{B,P,S}$	$+^{P}$	$++^{B,P,S}$	$++^{B,P,S}$
LUNG PARENCHYMA	$+++^{S,V}$	−	$++^{S,V}$	$+^{S,V}$
GASTROINTESTINAL				
ESOPHAGUS	$+++^{B,V,P,S}$	$+^{P}$	$++^{V,P,S}$	$++^{B,V,P,S}$
STOMACH	$+++^{B,V,P}$	−	$++^{V,P}$	$++^{B,V,P}$
SMALL INTESTINE	$++^{B,P,S}$	−	$++^{P}$	$+^{B,P}$
LARGE INTESTINE	$+++^{B,V,P,S,M}$	$+^{P}$	$+^{V,P,S,M}$	$++^{B,V,P,S,M}$
LIVER	$+++^{V}$	−	$+^{V}$	$+^{V}$
PANCREAS	$+++^{B,V,S,M}$	‡$+^{V}$	$++^{V,M}$	$+^{B,V,M}$
GALL BLADDER	$+++^{B,V,P,S}$	$+^{P}$	$+++^{V,P,S}$	$+++^{V,P,S}$
GENITOURINARY				
GLOMERULI	$+++^{V,S}$	−	$+^{V,S}$	$++^{V,S}$
TUBULES/INTERSTITIUM	$+++^{B,V,S}$	−	$++^{B,V,S}$	$++^{B,V,S}$
BLADDER/URETERS	$+++^{B,V,P,S}$	$++^{P,S}$	$++^{V,P,S}$	$++^{B,V,P,S}$
§OVARY	$+++^{V,S}$	$+^{V,S}$	$+^{V,S}$	$++^{V,S}$
§OVIDUCT	$+++^{V,P,S}$	$++^{P,S}$	$++^{V,P,S}$	$++^{V,P,S}$
§UTERUS	$+++^{B,V,P,S}$	$++^{B,V,P,S}$	$++^{B,V,P,S}$	$+++^{B,V,P,S}$
§VAGINA	$+++^{B,V,P,S}$	$+^{P}$	$+^{B,V,P,S}$	$++^{B,V,P,S}$
TESTIS	$+++^{B,V,M}$	−	$+^{B,V,M}$	$++^{B,V,M}$
MUSCULOSKELETAL				
SKELETAL MUSCLE	$+++^{V,P}$	‡+	$++^{V,P}$	$+^{V,P}$
HYALINE CARTILAGE	+++	¥++	−	+++
SYNOVIUM	$+++^{S}$	$++^{S}$	−	$++^{S}$
HEMATOPOIETIC				
SPLEEN	$+++^{V}$	−	$+^{V}$	$++^{V}$
BONE MARROW	−	−	−	−
LYMPH NODE	$+++^{V}$	−	−	$++^{V}$
NERVOUS SYSTEM				
BRAIN	$+++^{V}$	‡$+^{V}$	$++^{V}$	$++^{V}$
CHOROID PLEXUS	$+++^{V,S}$	‡$+^{V,S}$	$+^{V,S}$	$+^{V,S}$
MENINGES	+++	+	+	+
PERIPHERAL NERVE	$+++^{V,P}$	−	$+++^{V,P}$	$++^{V,P}$
EYE				
CORNEAL STROMA	$++^{S}$	−	−	$+^{S}$
DESCEMETS MEMBRANE	$+++^{B}$	$+++^{B}$	$+^{B}$	$+++^{B.}$
IRIS	$++^{P}$	$+^{P}$	$+^{P}$	$+^{P}$
LENS CAPSULE	++	++	−	++
ENDOCRINE				
THYROID	$++^{B,V,S}$	−	$+^{V,S}$	$+^{B,V,S}$
ANTERIOR PITUITARY	$+++^{V}$	−	−	$++^{V}$
ADRENAL	$+++^{B,V,S}$	−	−	$++^{B,V,S}$
INTEGUMENTARY				
SKIN	$+++^{B,V,S,F}$	$+^{B,F}$	$++^{V,S,F}$	$++^{B,S}$

*The tissue distributions of the EIIIB segment, the EIIIA segment, the V95 subsegment, and the total pool of FN were analyzed via immunofluorescence microscopy using segment-specific or, in the case of total FN, anti-cellular FN antibodies. Fluorescence staining intensity was subjectively graded as: (−) negligible, (+) faint, (++) moderate, (+++) strong. Several major patterns of tissue staining were observed: B = basement membrane zone, V = capillary and small blood vessels, P = perimysial/epimysial or, in the case of peripheral nerve perineurial/epineurial, S = subepithelial connective tissue, M = mesothelial, F = hair follicle sheath. †The hyaline cartilage of the trachea and bronchi is considered under "hyaline cartilage". ‡N-glycanase did not produce enhancement in the anti-EIIIB interstitial staining pattern. §The phase of the estrous cycle was not determined. ¥Anti-EIIIB antibodies produced a peripheral pattern of staining.

lar intensity. In contrast, anti-EIIIA staining appeared to be selectively concentrated in the walls of the capillaries. Such staining for the EIIIA segment was evident as "ring" structures throughout the atrial and ventricular walls, giving a "Swiss cheese" appearance (Figure 1A). Myocardial interstitial staining by anti-V antibodies was faint but, similar to both anti-total FN and anti-EIIIA antibodies, occasionally included "ring" patterns, again suggesting capillary wall staining (not shown).

Although anti-EIIIB antibodies failed to produce endocardial or pericardial staining, they did produce some faint, frequently amorphous, interstitial myocardial staining. These patterns were present in the

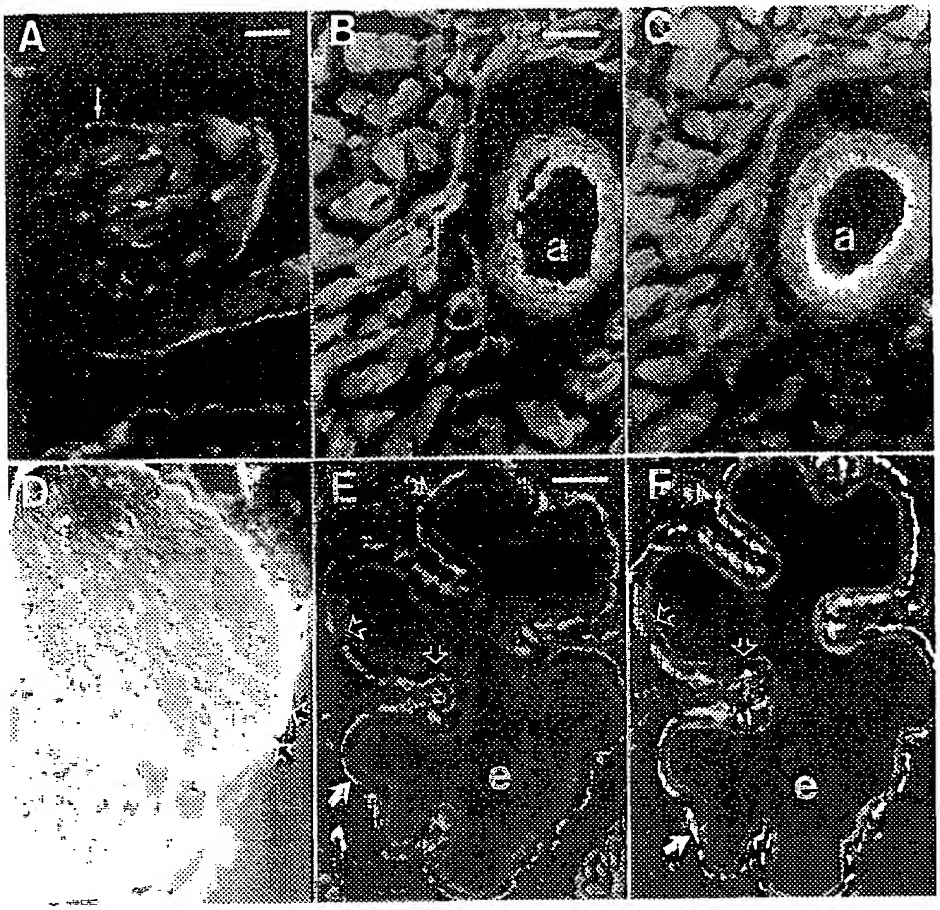

FIGURE 1 The EIIIA segment is widely distributed in the adult mouse. **Panel A**: A section through the atrium of a 15 week old mouse was stained with anti-EIIIA antibodies. Note the "ring" patterns of staining (small arrow), consistent with the walls of blood capillaries, in the atrial myocardium. The endocardium (long arrow) and pericardium are also stained. **Panels B and C**: Adjacent sections through an arteriole (a) within a skeletal muscle in the neck of a mouse aged 9 weeks 6 days, stained with **(B)** anti-EIIIA and **(C)** anti-V95 antibodies. Each antibody produces maximal staining within the intimal zone of the arteriolar wall, whereas only faint staining is present within the medial zone. The intimal staining pattern was not present when the primary antibodies were omitted, and therefore does not represent autofluorescence from the internal elastic lamina. In adjacent serial sections, anti-total FN antibodies produced a similar pattern of staining, whereas anti-EIIIB antibodies did not produce appreciable staining of the arterial wall (not shown). **Panel D**: A section through a peripheral nerve within the orbit of a mouse aged 9 weeks 6 days, stained with anti-EIIIA antibodies. In addition to the perineurium, there are also "ring" (arrow), and finer reticular/linear patterns of staining within the nerve. Anti-total FN and anti-V95 antibodies produced similar staining patterns in adjacent sections, but anti-EIIIB antibodies failed to produce staining (not shown). **Panels E and F**: Adjacent transverse sections through the esophagus (e) of a mouse aged 8 weeks 6 days, stained with **(E)** anti-EIIIA and **(F)** anti-V95 antibodies, respectively. Each antibody produces staining of smooth muscle (solid arrows) and loose subepithelial connective tissue, with associated blood vessels. Note, however, that the anti-V, but not anti-EIIIA, antibodies also produce distinct staining of the BMZ immediately beneath the squamous epithelium (open arrows). In adjacent sections, anti-total FN antibodies produced staining which was similar to that produced by anti-V antibodies, whereas anti-EIIIB antibodies produced a pattern of staining which was similar, but fainter, than that produced by anti-EIIIA antibodies. Bar, 20 μm in A, 50 μm in B-D, and 100 μ in E and F.

absence of N-glycanase pretreatment of sections. Although they may, in certain instances, have represented specific staining of capillary wall and/or endomysial FNs, in most cases such staining could be traced to peripherally-stained intravascular RBCs.

Anti-total FN, anti-V, and anti-EIIIA antibodies, in order of intensity, each produced staining within the atrioventricular and semilunar valves. The first two antibodies produced fibrillar staining in the substance of the valves. In contrast, anti-EIIIA antibodies produced staining at the surfaces of cells within the valvular stroma, but did not produce fibrillar staining between such cells. Anti-EIIIB antibodies failed to produce staining of the valves (not shown).

Blood Vessels. In the thoracic aorta, anti-total FN, anti-EIIIA, and anti-V antibodies (in order of intensity) each produced bright fibrillar staining of the extracellular material lying between the elastic laminae of the media, as well as within the intimal zone (Figure 2). The intimal staining produced by anti-total FN and anti-V antibodies tended to be more concentrated than the adjacent medial staining. It was also usually more continuous in quality than the intimal staining produced by anti-EIIIA antibodies. In contrast, no reproducible staining of the aortic media or intima was obtained with anti-EIIIB antibodies (Figure 2).

In the walls of smaller muscular arteries and arterioles, anti-EIIIA, anti-V, and anti-total FN antibodies each typically produced concentrated intimal staining, in addition to fainter fibrillar staining of the media (Figure 1, panels B and C). Such intimal staining was localized to the lumenal side of the internal elastic lamina. B $A^{+}V^{+}$ staining patterns similar to those shown in Figure 1 were observed in the walls of small arteries in the myocardium (coronary arteries), pancreas, testis, and spleen (not shown). Although anti-EIIIB antibodies frequently failed to produce staining within arterial walls, some faint N-glycanase-enhanceable fibrillar staining for the EIIIB segment was detected in the media of the lobar and arcuate branches of the renal arteries (not shown). Faint staining for EIIIB+ FN was also detected in the walls of meningeal arterioles as well as small arteries within both the nongravid and gravid uterus (see Figure 5, top two rows).

The patterns of staining that were produced by the anti-FN antibodies in capillary structures are discussed below by organ system.

Respiratory System. Although epithelial cells of the respiratory tract lacked anti-FN staining, the basement membrane zone (BMZ), subepithelial connective tissue (lamina propria), and smooth muscle of the

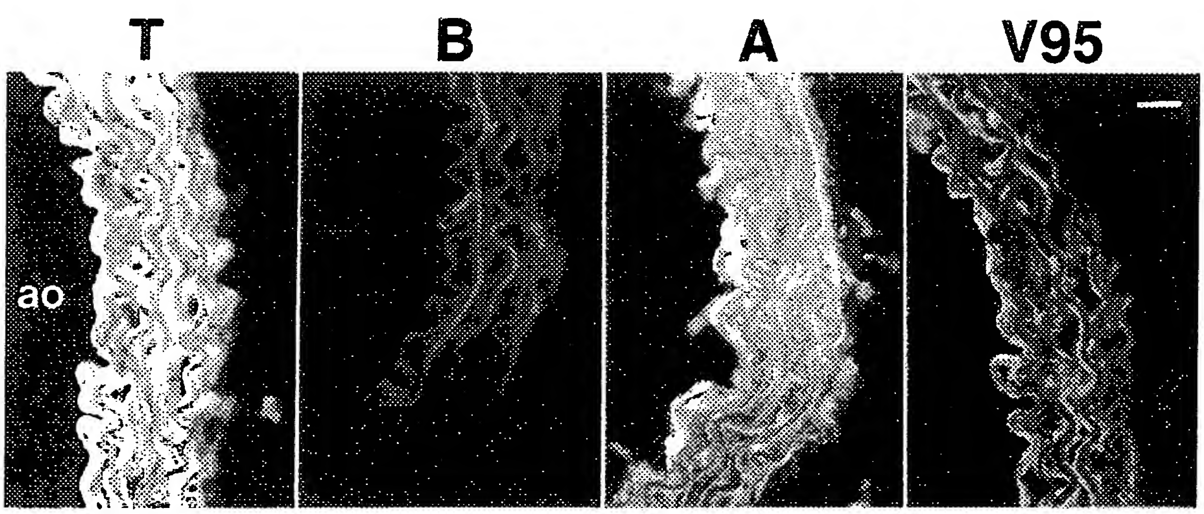

FIGURE 2 Differential distribution of the alternatively spliced segments of FN in the adult mouse aorta. Closely situated serial tranverse sections through the thoracic aorta of a mouse aged 8 weeks 6 days were subjected to staining, proceeding from left to right, with anti-total FN, anti-EIIIB, anti-EIIIA, and anti-V antibodies. Anti-total FN, anti-EIIIA, and anti-V antibodies each produced staining of pericellular material lying between the elastic laminae in the wall of the aorta. In contrast, anti-EIIIB antibodies produced negligible staining, although patches of anti-EIIIB staining were occasionally observed at the lumenal surface (not shown). Bar, 20 μm.

trachea and bronchi were each stained by anti-total FN, anti-EIIIA, and anti-V antibodies, in decreasing order of intensity (not shown). Each of these three antibodies also produced staining of the walls of the pulmonary arteries and veins, the alveolar septae, and the pleural membranes, in the same order of intensity (Figure 3). The staining produced by anti-total FN and anti-V antibodies did not appear to differ significantly in intensity between the alveolar interstitium and the walls of larger pulmonary blood vessels, whereas anti-EIIIA antibodies produced more intense staining of large blood vessel walls than of septal structures (Figure 3). Although it was not possible to identify the alveolar structural components that were stained by each of the anti-FN antibodies in our frozen sections, the patterns of septal staining that were produced by anti-EIIIA and anti-V antibodies appeared to be qualitatively similar to one another and to those produced by anti-total FN antibodies (Figure 3). Anti-EIIIB antibodies failed to produce appreciable staining of the trachea, bronchi, interstitium, or pleura (Figure 3,B). However, these antibodies did produce faint staining of smooth muscle associated with bronchi.

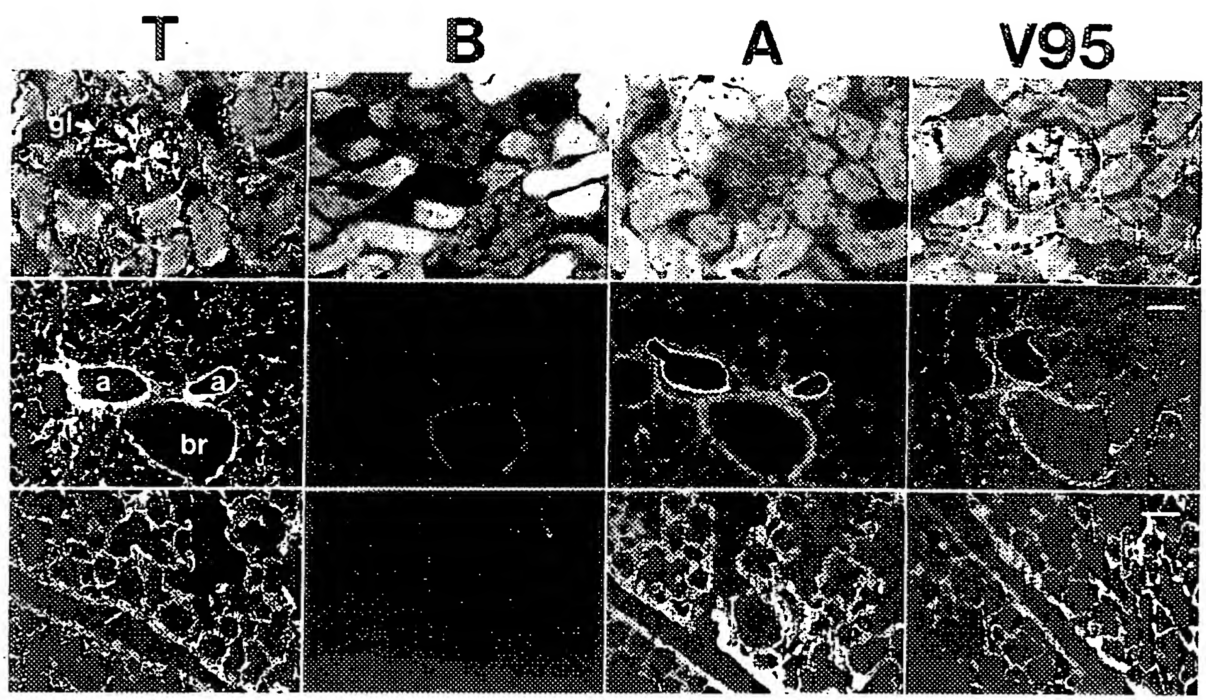

FIGURE 3 Differential distribution of the alternatively spliced segments of FN within the microvasculature of the adult mouse. Tissue sections from adult mice were stained, proceeding from left to right, with anit-total FN, anti-EIIIB, anti-EIIIA, and anti-V antibodies. **Top row**: Sections through the kidney of a 15 week old mouse. Anti-total FN and anti-V antibodies each produce staining of the glomerular (gl) mesangium and capillaries, the parietal epithelium, and the tubular interstitium. No staining by anti-EIIIB antibodies was evident in either of two glomeruli shown, or in the tubular interstitium. **Middle row**: Closely situated serial sections through adult mouse lulng, showing a bronchiole (br) and an arteriole (a), the latter of which is transected twice. Anti-total FN, anti-EIIIA, and anti-V antibodies each produce fibrillar staining of the alveolar interstitium, the bronchiolar BMZ, and the arteriolar walls. Notably, in comparison with anti-total FN and anti-V antibodies, anti-EIIIA antibodies appear to produce more intense staining of the arteriolar walls as opposed to other pulmonary structures. Anti-EIIIB antibodies produce little staining of adult lung. Although some staining of bronchial epithelial cells is apparent in each of the four panels, this is not specific for FN, since it was also present in the absence of primary antibodies (not shown). **Bottom row**: Higher magnification of the lung parenchyma. In addition to the alveolar interstium, anti-total FN, anti-EIIIA, and anti-V antibodies each produce staining of the pleural membrane (arrow). The sections shown in the middle and bottom rows were obtained from an unfixed lung that had been frozen after inflation with embedding medium via the trachea (see Methods). Similar patterns of staining were observed in lungs that had been fixed by the usual method (not shown). Bar, 20 μm in top row, 100 μm in middle row, and 50 μm in bottom row.

Gastrointestinal System

Oropharynx. None of the four anti-FN antibodies produced appreciable staining of the cells of the stratified squamous epithelium of the tongue. However, the BMZ and lamina propria underlying the epithelium were each stained brightly by anti-total FN and, to a lesser extent, by anti-V antibodies (Figure 4). Some faint staining of the lamina propria was also produced by anti-EIIIA antibodies, but anti-EIIIB staining was negligible.

Gastrointestinal Tract. Although anti-EIIIB antibodies produced some faint fibrillar staining of smooth muscle surrounding the esophagus and large

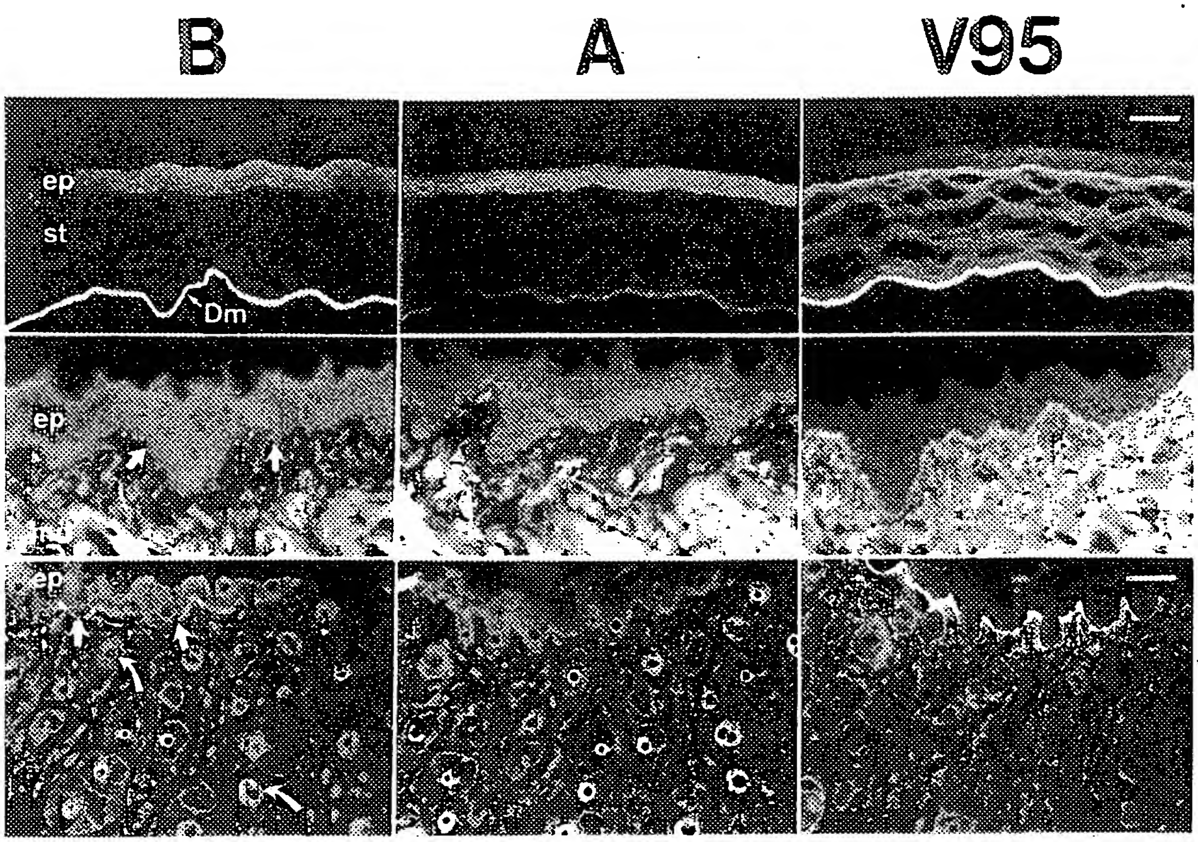

FIGURE 4 Differential distribution of the alternatively spliced segments of FN in adult mouse epithelia. Closely situated serial sections through adult mouse epithelia were subjected to staining, proceeding from left to right, with anti-EIIIB, anti-EIIIA, and anti-V antibodies. **Top row**: Sections through the cornea of a mouse, aged 15 weeks, showing the epithelium (ep), stroma (st), and Descemet's membrane (Dm). Descemet's membrane is stained brightly by anti-EIIIB, anti-V, and anti-total FN antibodies (latter not shown), whereas anti-EIIIA antibodies produce only faint staining of this structure. Anti-EIIIB and anti-EIIIA antibodies both produce negligible staining of the corneal stroma, whereas anti-total FN antibodies produce diffuse stromal staining (not shown). Anti-V antibodies produce stromal staining that is restricted to the zone immediately beneath the epithelium. **Middle row**: Sections through the tongue of a mouse, aged 9 weeks 6 days, showing (proceeding from top to bottom) epithelium (ep), BMZ (arrows), subepithelial connective tissue (lamina propria), and skeletal muscle fibers (mu). Anti-V antibodies (and anti-total FN antibodies, not shown) produce abundant staining of the BMZ and lamina propria, whereas anti-EIIIB and anti-EIIIA antibodies produce little staining of these structures. Nonspecific background fluorescence is apparent within the muscle fibers at the bottom of the panels. **Bottom row**: Sections through the skin of the snout of a mouse, aged 8 weeks 6 days. Note that each of the antibodies produces negligible staining of the epidermis (ep). Anti-V antibodies produce strong staining of the BMZ (denoted by straight arrows in left panel only) and subjacent connective tissue, but fail to produce much staining of hair follicles (curved arrows). In contrast, anti-EIIIB antibodies give patches of faint staining of the BMZ as well as intermittent staining of hair follicle sheaths. Anti-EIIIA antibodies produce negligible basement membrane zone staining, but stain most hair follicle sheaths. Bar, 50 μm in top two rows, 100 μm in bottom row.

intestine, they failed to produce appreciable staining of the visceral smooth muscle associated with the stomach or small intestine. In contrast, anti-total FN, anti-V, and anti-EIIIA antibodies each produced distinct staining of the smooth muscle and subepithelial connective tissue of the gut at all levels (Figure 1). In general, anti-total FN antibodies produced the most intense staining of such visceral smooth muscle, followed by anti-V and anti-EIIIA antibodies, which showed similar intensities. Additionally, anti-total FN and anti-V antibodies each produced staining of the epithelial BMZ at all levels of the gut, whereas anti-EIIIB and anti-EIIIA antibodies typically failed to produce such staining (Figure 1). We also detected staining of the mesothelium of the large intestine with anti-total FN, anti-EIIIA, and and anti-V, but not anti-EIIIB antibodies.

Liver and Gall Bladder. Consistent with several previous studies in which FN expression has been analyzed at the mRNA level (Schwarzbauer et al., 1987; Oyama et al., 1989; Pagani et al., 1991; Jarnagin et al., 1994), the parenchyma of the adult liver was largely negative for staining for both the EIIIB and EIIIA segments, although we did detect $EIIIA^+$ FN in the walls of portal arterioles. In contrast, anti-total FN and anti-V95 antibodies each produced staining of the walls of central veins and sinusoids, in addition to portal blood vessels. The vascular staining produced in the liver by anti-V95 antibodies was considerably fainter than that produced by anti-total FN antibodies (not shown).

In the gall bladder, anti-total FN, anti-EIIIA, anti-V, anti-EIIIB antibodies, in order of intensity, each produced similar patterns of fibrillar staining of the smooth muscle (not shown). The first three antibodies also produced staining of the subepithelial stroma and of the walls of associated blood vessels.

Pancreas. Anti-total FN, anti-EIIIA, anti-V, and anti-EIIIB antibodies, in order of intensity, each produced staining of the walls of small interstitial tubular structures that appeared to conform with capillaries. The capillary staining produced by anti-EIIIB antibodies was N-glycanase-independent, but could be blocked specifically by EIIIB-GST fusion protein. The walls of small interstitial arteries were also stained by anti-total FN, anti-EIIIA, and anti-V (but not by anti-EIIIB) antibodies. The epithelial BMZ and subepithelial connective tissue of the main pancreatic ducts were stained by anti-total FN and anti-V, but not anti-EIIIB or anti-EIIIA, antibodies. Only anti-total FN antibodies produced appreciable staining in the BMZ beneath the acinar cells, and this was patchy and faint. The mesothelium was stained by anti-total FN, anti-V, and anti-EIIIA antibodies, in order of intensity (not shown).

Genitourinary System

Kidney. In the glomerulus, anti-total FN and anti-V antibodies each produced staining of the mesangium and, to a lesser degree, capillaries. In comparison, anti-EIIIA antibodies produced very faint staining which appeared to be restricted to the mesangium of the glomerular stalk and to the walls of afferent and efferent arterioles (Figure 3). The parietal epithelium was also stained by anti-total FN, anti-V, and to a much lesser extent, anti-EIIIA antibodies (Figure 3). The same three antibodies (same order of intensity) also produced staining of the tubular BMZ and interstitium. Anti-EIIIB antibodies failed to produce appreciable staining of either the glomeruli or the interstitium. Small vessels of the vasa recta within the medullary pyramid were $B^-A^+V^+$ (not shown).

Ureters and Bladder. The cells of the transitional epithelium of the urinary tract lacked staining for FN. However, each of the segment-specific antibodies produced staining of subepithelial connective tissue and smooth muscle of the ureters and bladder. However, only anti-total FN and anti-V antibodies produced staining of the BMZ of the bladder epithelium.

Female Reproductive Tract. Anti-total FN, anti-V, anti-EIIIA, and anti-EIIIB antibodies, in order of intensity, each produced staining of the smooth muscle and subepithelial connective tissue stroma of the vagina (not shown) and uterus (Figure 5), as well as of the interstitial stroma and blood vessels of the ovaries

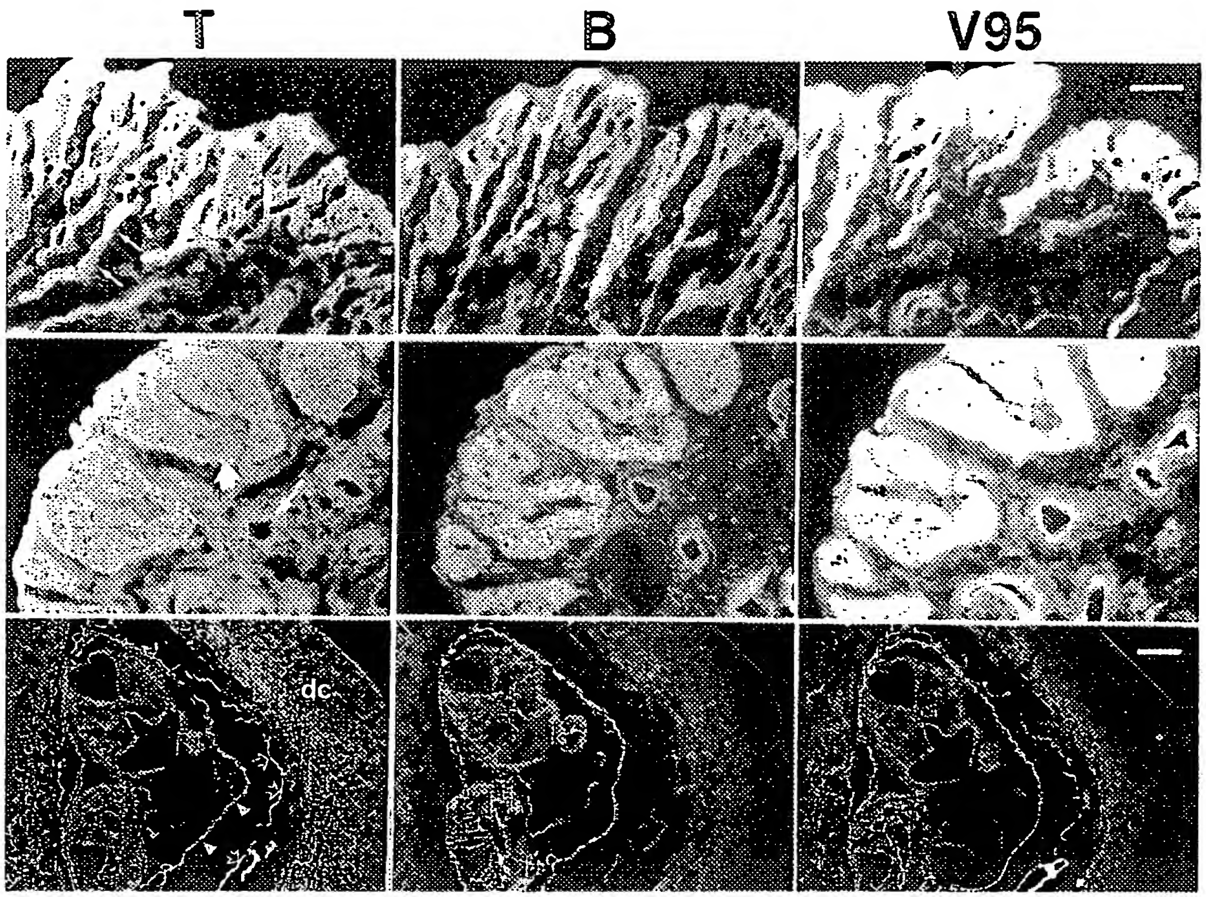

FIGURE 5 Uterine distribution of the spliced segments of FN. Closely situated tissue sections of adult mouse uterus were subjected to staining, from left to right, with anti-total FN, anti-EIIIB, and anti-V antibodies. Anti-EIIIA antibody staining was similar in each instance to that produced by anti-EIIIB antibodies (not shown). **Top row**: Sections through the external coat of myometrial smooth muscle of a non-gravid uterus, showing muscle fibers cut longitudinally (arrows). **Middle row**: Sections through a gravid uterus at 9.5 days gestation, showing myometrial muscle fiber bundles sectioned transversely (thick arrow). Each antibody produces endomysial staining, as well as staining of the walls of uterine blood vessels (thin arrow). **Bottom row**: Additional sections through the gravid uterus shown in the middle row, including an embryo. Although each antibody produces similar patterns of embryonic staining, different patterns are evident in the decidua capsularis (dc). Here, anti-total FN antibodies produce a vascular pattern of staining throughout the uterine wall, which is greatest in the inner trophoblastic meshwork. Anti-V antibodies produce a fainter vascular pattern, restricted primarily to the inner trophoblastic zone. In contrast, little or no staining of the decidua capsularis is produced by anti-EIIIB (or anti-EIIIA, not shown) antibodies. Note also that anti-total FN and anti-V antibodies both produce bright staining of the detached parietal layer of the yolk sac (open arrows), whereas anti-EIIIB antibodies produce relatively faint staining. In contrast, each of the antibodies produces intense staining of the visceral layer of the yolk sac (arrowheads). Bar, 50 μm in the top two rows, 200 μm in bottom row.

(not shown). The first three antibodies, in the same order of intensity, also produced epithelial BMZ staining within the vagina and uterus. Anti-EIIIB antibodies failed to produce appreciable vaginal BMZ staining, but did produce some faint staining of the epithelial BMZ in the uterus (not shown). In the oviducts, each of the four antibodies produced prominent staining of smooth muscle, as well as fainter staining of the subepithelial stroma and blood vessels (not shown).

Within the gravid uterus at 7.5 days gestation (Figure 5, second row), staining for the EIIIB, EIIIA, and V segments was prominent within the myometrium and in the walls of dilated endometrial

blood sinuses localized near the mesometrium (also see Peters and Hynes, 1996, this issue). In contrast, very little staining for the three spliced segments was detected between the inner circular layer of myometrial smooth muscle and the yolk sac cavity, despite diffuse staining of small interstitial blood vessels within this central zone by anti-total FN antibodies (not shown). By 9.5 days gestation, staining for the EIIIB segment was absent both from the uterine decidua and from the walls of dilated maternal blood sinuses at the mesometrial aspect of the uterus. Staining for the EIIIA segment was also absent from the decidua, but persisted in the walls of the maternal sinuses. Anti-total FN antibodies, and to lesser extent anti-V antibodies, produced abundant vascular staining at each of these sites, including the decidua capsularis (Figure 5, bottom row). The placental pattern produced by each of the four anti-FN antibodies at 9.5 days gestation has been described (Peters and Hynes, 1996, this issue).

Male Reproductive Tract. The capsule of the testis was stained by anti-total FN, anti-V, and anti-EIIIA antibodies, in order of intensity. These three antibodies also stained the BMZ surrounding the seminiferous tubules, and the walls of subcapsular arteries. In contrast, anti-EIIIB antibodies failed to produce staining (not shown).

Musculoskeletal System

Skeletal Muscle. The patterns of segment-specific and total FN immunostaining that were observed in the tongue and diaphragm (Figure 6), as well as intercostal (Figure 7), paraesophageal, extraocular (Figure 6), and laryngeal muscles, were generally similar to one another, and to cardiac muscle (see above). Typically, anti-total FN antibodies produced the brightest staining, followed next by antibodies to the EIIIA segment. In sections cut longitudinally, anti-total FN and anti-EIIIA antibodies generally produced similar linear patterns at the periphery of muscle fibers and within the perimysial stroma. However, in transverse sections through fibers, anti-total FN antibodies produced intense staining of both endomysium and capillaries (the latter were seen as punctate or "ring" patterns), whereas anti-EIIIA antibodies appeared to produce more staining of capillary structures than of endomysium (Figure 6).

There was a sharp reduction in the intensity of staining for the V and EIIIB segments in skeletal muscle of the adult mouse as compared with the embryo (Figures 6 and 7). Nevertheless, anti-V antibodies produced faint staining of the interstitium of adult skeletal muscles, which in certain cases included "ring" patterns consistent with capillary structures (not shown). The staining produced in adult skeletal muscle by anti-EIIIB antibodies was extremely faint and patchy, and was not enhanced by N-glycanase. In certain cases, it included "ring" patterns that could be traced to circulating RBCs, rather than to capillaries or other tissue structures.

Cartilage. Consistent with several previous studies in which FN mRNA encoding the EIIIB segment has been detected in mature hyaline cartilage (Bennett et al., 1991; Magnuson et al., 1991; Zhang et al., 1995), we found staining for this segment in several hyaline cartilage structures in the adult mouse. Unlike the patterns observed in the mouse embryo (Figure 7, top two rows), the staining for this segment in laryngeal, tracheal, bronchial, and costochondral cartilages was found to differ from that produced by antibodies to the V segment or to the total pool of FNs. Anti-V and anti-total FN antibodies stained the matrix and chondrocytes throughout these structures (Figure 7, bottom two rows), whereas anti-EIIIB antibodies produced staining that appeared to be restricted to peripherally-situated matrix, cells, and perichondrium (Figure 7, bottom two rows). Consistent with the pattern established during late embryogenesis (Figure 7, second row), adult hyaline cartilage lacked staining by anti-EIIIA antibodies (Figure 7, third row).

Anti-total FN, anti-EIIIB, and anti-V antibodies, in order of intensity, each produced bright staining of the synovial lining of the cricoarytenoid joint. In contrast, anti-EIIIA antibodies produced little or no staining (Figure 7, bottom row).

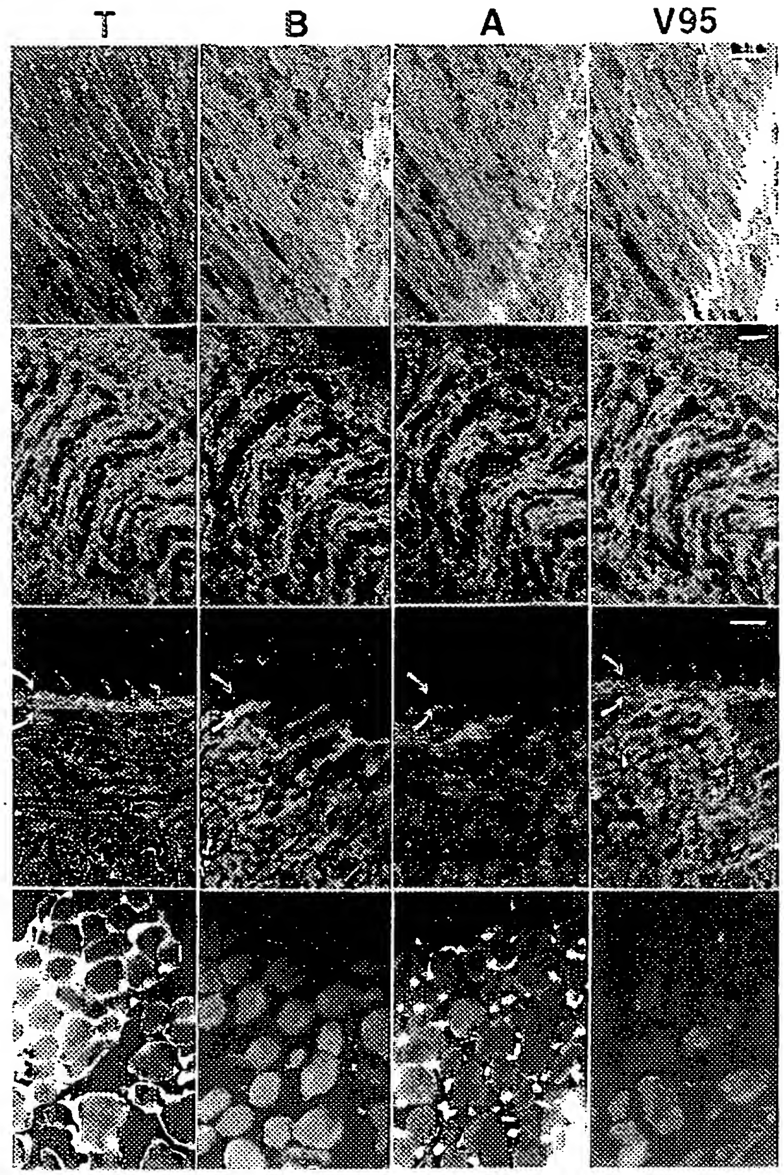

FIGURE 6 Differences in muscular expression of the alternatively spliced segments of FN between embryonic and adult mice. Nearby tissue sections were stained, from left to right, with anti-total FN, anti-EIIIB, anti-EIIIA, and anti-V antibodies. **Top row**: The diaphragm of a 16.5 day mouse embryo, sectioned longitudinally with respect to muscle fibers. **Second row**: Sagittal sections through the tongue of an embryo at 15.5 days gestation. **Third row**: Sagittal sections through the tongue of a mouse aged 9 weeks 6 days, showing the epithelium with filiform papillae above and skeletal muscle below. As described in Figure 4, the lamina propria (lying between the tips of the curved arrows) is largely $B^-A^-V^+$. Anti-total FN antibodies produce a predominantly linear pericellular pattern of staining within the underlying skeletal muscle. In contrast, anti-EIIIA staining appears to be primarily punctuate in areas in which muscle fibers are cut in cross-section, and primarily linear in areas cut longitudinally. Little muscular staining is evident with either anti-EIIIB or anti-V antibodies, despite greater exposure of the respective photos. **Bottom row**: Closely situated transverse sections through extraocular muscle of a mouse aged 15 weeks. Anti-total FN antibodies produce homogeneous staining that includes endomysial sheaths and interstitial densities conforming with capillary structures. In contrast, anti-EIIIA antibodies produce a more punctuate pattern that appears to be concentrated in capillaries, rather than the endomysium. Anti-EIIIB and anti-V antibodies produce little staining, although the latter antibodies were typically observed to produce faint staining of both capillary and endomysial structures within striated muscles. Monoclonal antibody 3E2 was used to detect the EIIIA segment in embryonic muscle (top two rows), whereas G153 was used for adult muscle (bottom two rows). G141 was used to detect total FN in extraocular muscle only (bottom row). Bar, 50 μm in top row, 20 μm in second and bottom rows, and 100 μm in third row.

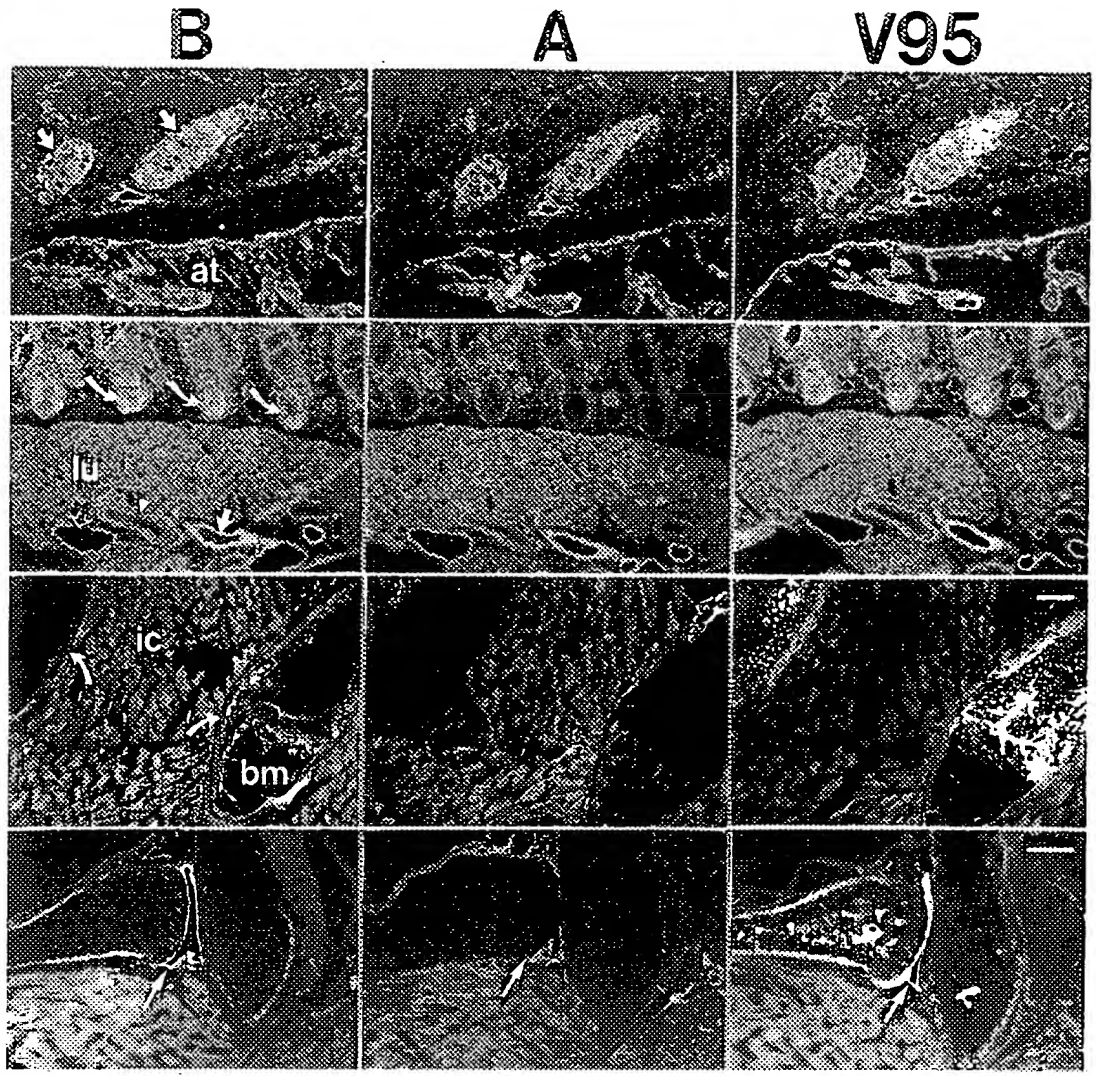

FIGURE 7 Differences in cartilaginous expression of the alternatively spliced segments of FN between embryonic and adult mice. Closely situated tissue sections were stained, from left to right, with anti-EIIIB, anti-EIIIA, and anti-V antibodies. The top two rows show sections from mouse embryos, whereas the bottom two rows show sections from an adult mouse aged 9 weeks 6 days. **Top row**: Sections through two condensations of cells at the sites of future ribs (arrows) and the adjacent atrium (at) at E12.5. Note that each of the three antibodies produces similar patterns of staining of the precartilaginous cellular condensations, in addition to the atrial wall. **Second row**: Sections through costal cartilages (curved arrows) and lung (lu) at E16.5, showing pulmonary artery (arrow), bronchus (arrowhead), and pulmonary vein (open arrow). Note that at this later point in development there is a selective absence of staining of cartilage by anti-EIIIA antibodies, but similar distributions of cartilaginous staining for the EIIIB and V segments. **Third row**: Sections through two costal cartilages (curved arrows) of an adult mouse. The rightmost of the two cartilages terminates inferiorly in bone, containing a marrow cavity (bm). Similar to the case for the late embryo, anti-EIIIA antibodies fail to stain costal cartilage in the adult mouse, whereas anti-EIIIB and anti-V antibodies continue to stain the matrix and chondrocytes. However, the pattern of staining produced by anti-EIIIB antibodies in adult cartilage appears to be more peripheral than that obtained with anti-V antibodies (and anti-total FN antibodies, not shown). Also note that the intervening intercostal muscle (ic) is stained by anti-EIIIA antibodies in a punctuate capillary-like pattern, whereas anti-EIIIB and anti-V antibodies produce minimal muscular staining. None of the anti-FN antibodies produce appreciable staining within the bone marrow cavity. **Fourth row**: Sections through the cricoarytenoid joint (arrow) in the adult mouse larynx. Anti-EIIIB and anti-V antibodies produce intense staining of the synovial lining of the joint space, whereas anti-EIIIA antibodies produce little synovial staining. In the hyaline cartilage on each side of the joint, anti-EIIIB antibodies produce a peripheral pattern which appears to include perichondrium, anti-EIIIA antibodies produce little or no staining, and anti-V (and anti-total FN, not shown) antibodies produce diffuse staining. Monoclonal antibody 3E2 was used to detect the EIIIA segment in embryonic cartilage structures (top two rows), whereas G153 was used for adult tissues (bottom two rows). Bar, 100 μm in top and fourth rows, 200 μm in second and third rows, and 50 μm in bottom row.

Hematopoietic System

Bone Marrow. None of the anti-FN antibodies produced appreciable staining of bone marrow obtained from the femur of an adult mouse.

Spleen. Anti-total FN and, to a lesser degree, anti-V95 antibodies produced staining of the capsule, the trabeculae, and the reticular network of fibers present throughout the organ. Staining of this reticular network was most distinct against the "dark background" of the white pulp, although the network itself appeared to be more dense in the red pulp. Anti-EIIIA antibodies also produced staining of the capsule, trabeculae, and reticular fibers, but very faintly. Anti-total FN, anti-V95, and anti-EIIIA antibodies each produced intimal staining in the walls of arteries within the white pulp. Anti-EIIIB antibodies failed to produce staining of splenic tissue.

Lymph Node. In paratracheal lymph nodes of a mouse aged 15 weeks, anti-total FN antibodies produced staining of the capsule, the walls of blood vessels, and the reticular network of fibers. As described previously (De Pasquale et al., 1986), such reticulae appeared to be more prevalent in the medulla than in the cortex. Anti-V antibodies produced a similar, though slightly fainter pattern. In contrast, anti-EIIIB and anti-EIIIA antibodies produced little or no staining of paratracheal lymph nodes (not shown).

Nervous System

Brain and Meninges. Consistent with previous studies, the neuronal and glial cellular components of the brain failed to show specific staining for FN (Hynes, 1990). However, each of the anti-FN antibodies produced vascular, meningeal, and ependymal staining. In general, anti-V and anti-EIIIA antibodies produced more intense staining of larger meningeal and parenchymal blood vessels and relatively less intense staining of intraparenchymal and choroid plexus capillaries, whereas anti-EIIIB antibodies appeared to have the opposite preference (Figure 8).

The staining produced by anti-EIIIB antibodies in the meninges, walls of meningeal blood vessels, and ependyma was N-glycanase-dependent. In contrast, the staining produced in capillary-sized vessels within the brain and choroid plexus was not enhanced by such treatment (Table I and Figure 8), raising the possibility that peripherally-stained deformable RBCs (see above) might account for some "capillary" patterns. In fact, fluorescently stained RBCs could in certain instances be visualized within large and small blood vessels in the brain (Figure 8). However, we also observed capillary staining that appeared not to be RBC-dependent. For example, anti-EIIIB antibodies produced N-glycanase-independent staining of choroid plexus capillary walls while failing to produce appreciable staining within the capillary lumens, arguing against RBC-dependent staining (Figure 8). The meningeal staining produced by anti-EIIIB antibodies was N-glycanase-dependent and, like all of the staining patterns produced by these antibodies in the adult mouse brain, could be specifically blocked with EIIIB-GST fusion protein.

Peripheral Nerve. Anti-total FN, anti-EIIIA, and anti-V antibodies each produced staining of the perineurium (Figure 1). In transverse sections, each of the three antibodies also produced a reticular pattern consistent with the endoneurium and/or the basement membrane of Schwann cells (Paetau et al., 1980; Longo et al., 1984). In addition to the above, anti-total FN, anti-EIIIA, and anti-V antibodies (in order of intensity) each produced discrete "ring" patterns in transverse sections that appeared to conform with capillary structures (Figure 1). Anti-EIIIB antibodies did not produce appreciable staining (not shown).

Eye. Anti-total FN antibodies produced diffuse staining of the corneal stroma. In contrast, anti-V antibodies produced weak stromal staining that appeared to be restricted to the area immediately beneath the corneal epithelium (Figure 4). Anti-EIIIB and anti-EIIIA antibodies failed to stain the stroma. The endothelial basement membrane (Descemet's

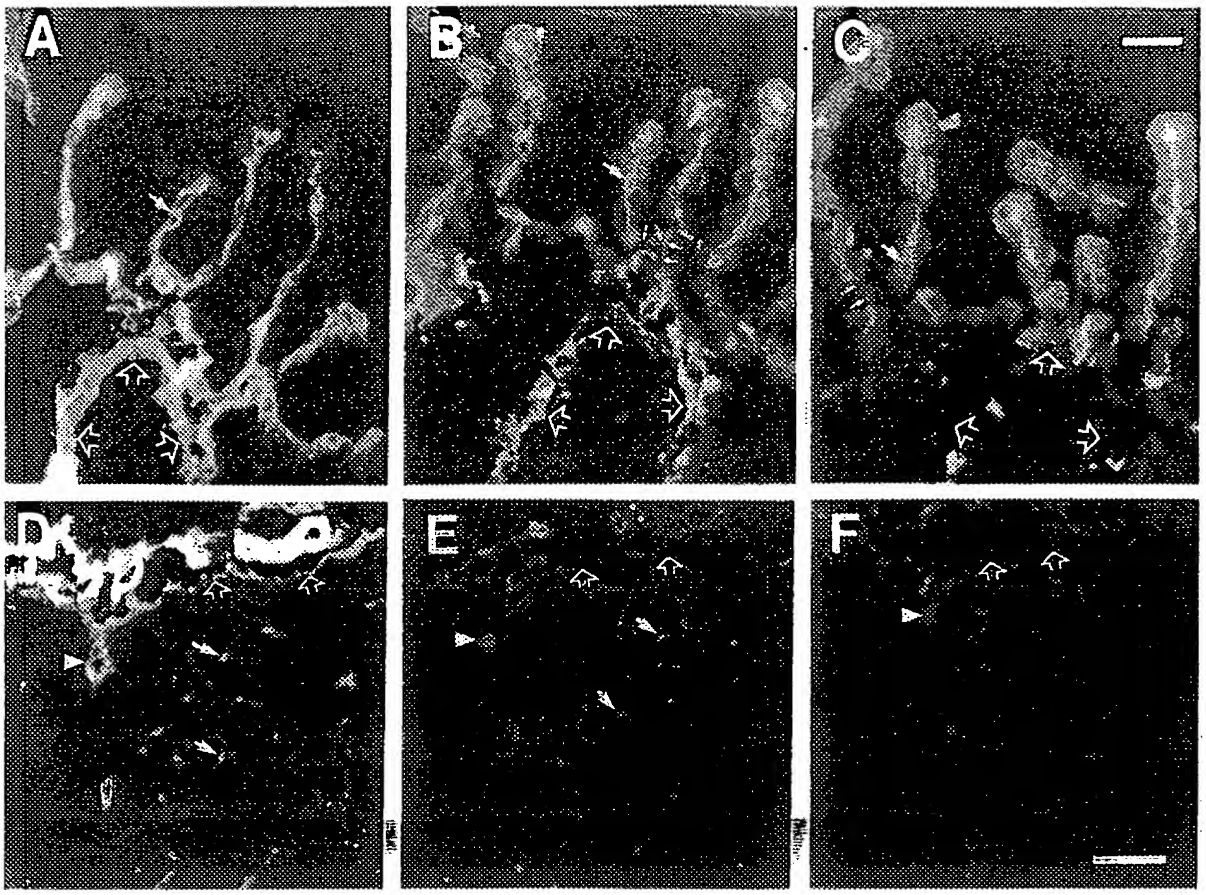

FIGURE 8 Anti-EIIIB antibodies produce N-glycanase-independent staining of capillaries in the nervous system. **Panels A-C**: Sections through choroid plexus of a mouse aged 9 weeks 6 days stained with **(A)** anti-total FN antibodies, **(B)** anti-EIIIB antibodies with, and **(C)** without N-glycanase pretreatment of the tissue section. Both anti-total FN and anti-EIIIB antibodies can be seen to produce linear and "ring" (solid arrow) patterns of staining in the center of choroid plexus fronds. Similar patterns were obtained with anti-A and anti-V antibodies (not shown), whereas none of the anti-FN antibodies produced appreciable staining of the cells of the choroid plexus epithelium. Note that, despite staining of the capillary walls by anti-EIIIB antibodies, stretches of capillary lumen appear to remain unstained (tiny arrows in **B and C**). Also note that deglycosylation is without apparent effect upon the capacity of anti-EIIIB antibodies to produce capillary-like staining in the choroid plexus (compare **B** and **C**), whereas anti-EIIIB antibody staining of the adjacent ependymal tissue (open arrows) is N-glycanase-dependent (compare **B** and **C**). **Panels D-F**: Sections through meningeal blood vessels (above) and brain (below), stained with **(D)** anti-total FN, **(E)** anti-EIIIB antibodies preincubated with EIIIA-GST, or **(F)** anti-EIIIB antibodies preincubated with EIIIB-GST fusion protein. Incubations were for 1 h with 286 μg fusion protein/ml. The sections shown in **(E)** and **(F)** had both been treated with N-glycanase prior to application of antibodies. Note that anti-total FN antibodies produce bright staining of meningeal blood vessels, pial membrane (open arrows), as well as intraparenchymal capillaries (solid arrows) and larger blood vessels (arrowhead). Anti-EIIIB antibodies, which produce substantially fainter staining of each of the above structures, can also be seen to produce faint staining of blood cells within a larger intraparenchymal blood vessel (arrowhead). Note that staining of all structures, including blood cells, was selectively abolished by preincubation of the anti-EIIIB antibodies with EIIIB-GST, but not EIIIA-GST, fusion protein. Bar, 20 μm in top panels, 50 μm in bottom panels.

membrane) was stained brightly by anti-total FN (not shown), anti-EIIIB, and anti-V antibodies, whereas anti-EIIIA antibodies produced little staining (Figure 4).

Anti-total FN, anti-EIIIA, and anti-V antibodies each produced staining of the walls of capillaries in the ciliary body, as well as of blood vessels in the choroid layer. In contrast, anti-EIIIB antibodies failed

to produce appreciable uveal staining. However, each of the three segment-specific antibodies produced faint staining of the ciliary muscle and the smooth muscle in the central iris (not shown).

None of the four antibodies produced staining of the anterior lens capsule, but the lateral and posterior capsule were stained by anti-total FN, anti-EIIIB, and anti-V, but not by anti-EIIIA antibodies. Retinal blood vessels, but not cells, were stained by all of the anti-FN antibodies.

Endocrine System

Thyroid. Anti-total FN antibodies produced staining of the interstitial capillaries and stroma, in addition to the follicular BMZ. A qualitatively similar, but fainter pattern of thyroid staining was produced by anti-V antibodies. In contrast, anti-EIIIA antibodies failed to stain the follicular BMZ, but did produce staining of perifollicular capillary structures. Anti-EIIIB antibodies produced negligible staining of the thyroid.

Pituitary. In the anterior pituitary, anti-total FN and, to a lesser extent, anti-V antibodies produced staining of capillary-like structures, whereas no appreciable staining was apparent with anti-EIIIB or anti-EIIIA antibodies.

Adrenal. Capsular staining was strong with anti-total FN antibodies, and faint with each of the three segment-specific antibodies. Anti-total FN antibodies produced staining in both the cortex and medulla. This appeared to include basement membranes surrounding cords of epithelial cells as well as the walls of vascular structures. A similar but fainter interstitial pattern was produced by anti-V antibodies, but anti-EIIIB and anti-EIIIA antibodies each failed to produce appreciable staining.

Skin. In skin obtained from the snout, anti-V antibodies produced prominent subepithelial and BMZ staining, but little staining of follicle sheaths. In contrast, anti-EIIIA antibodies failed to produce appreciable BMZ staining, but frequently stained the follicle sheaths. Anti-EIIIA antibodies also produced prominent staining of the walls of dermal blood capillaries. Anti-EIIIB antibodies produced some faint staining of the hair follicle sheaths, as well as intermittent faint BMZ staining (Figure 4).

DISCUSSION

Fibronectin is a widespread component of vertebrate tissues, serving in major capacities as both a soluble plasma protein and as a component of the insoluble ECM. It seems unlikely that such a biologically potent molecule serves exactly the same set of functions in all of its sites of expression. An obvious mechanism by which the functions of FN could be tailored to site-specific roles is through adjustments in primary structure via alternative splicing. Consistent with such a proposal, we have observed that the three alternatively spliced segments of FN show numerous quantitative and qualitative differences in distribution in tissues of the adult mouse. This situation differs strikingly from that in the early mouse embryo, in which the segments codistribute widely (Peters and Hynes, 1996, this issue; also see Figures 5 and 6).

Tissue Distribution of the EIIIB Segment

Among the three isoform-specific antibodies, those recognizing the EIIIB segment showed the greatest overall reduction in staining in the adult mouse as compared with the embryo. Nevertheless, staining for this segment was present in several adult tissues, potentially providing clues to its functions. For example, anti-EIIIB staining was observed to be restricted to peripheral zones in hyaline cartilage, despite diffuse cell and matrix staining by anti-total FN and anti-V antibodies. This represents the second instance of divergence in staining patterns produced by segment-specific anti-FN antibodies in hyaline cartilaginous structures during the life of the mouse. In the first, evident in mid-embryonic development and complete by E15.5, the $B^+A^+V^+$ splicing pattern of precartilaginous condensations (Figure 7, top row)

gives way to a $B^+A^-V^+$ staining pattern in structures undergoing overt chondrogenesis (Figure 7, second row; Peters and Hynes, 1996, this issue). In both of these previous stages, staining for the EIIIB and V segments was observed to colocalize. In contrast, B^+ FNs appear to represent a minor, spatially-restricted population within a larger pool of A^-V^+ FNs in adult hyaline cartilage (Figure 6, bottom two rows). This is consistent with the previous observation that various adult canine cartilages contain both B^+ and B^- FN mRNAs, with B^+ transcripts constituting 17 to 30% of the total (Zhang et al., 1995). Furthermore, our observations suggest that the cells and matrix at the periphery of mature hyaline cartilages contain $B^+A^-V^+$ FN, whereas the FN that is expressed in the internal matrix and cells is $B^-A^-V^+$. Potentially, inclusion of the EIIIB segment might serve to generate an ECM substrate that is facilitative for proliferation (ffrench-Constant and Hynes, 1989) and/or inhibition of terminal differentiation of primordial cells located in the perichondrium or the peripheral zones of such cartilage. Such a "pro-growth" role would appear to be ideally suited to the EIIIB segment since, as mentioned above, it appears by immunostaining to be the most tightly developmentally regulated component of FN.

Bright staining by anti-EIIIB antibodies was also present in the endothelial (Descemet's) basement membrane of the adult cornea. This morphologically unique basement membrane is composed of a highly ordered hexagonal network which includes type VIII collagen (Kapoor et al., 1986; Sawada et al., 1990). The staining produced in this structure by anti-EIIIB antibodies raises the possibility that inclusion of the EIIIB segment could affect the capacity of FNs to engage in interactions with specific collagenous matrices or collagens, e.g. type VIII. A characteristic roster of collagens (types II, IX, X, and XI) is also known to assemble in hyaline cartilage (Thomas et al., 1994). Among these, type X collagen has the capacity, at least *in vitro*, to form hexagonal matrices (Kwan et al., 1991). Thus, it is possible that two of the adult tissue structures (hyaline cartilage and Descemet's membrane) in which we have detected a $B^+A^-V^+$ pattern include hexagonal networks of collagen. Higher resolution analyses of the disposition of the EIIIB segment in relation to such ordered matrices, as well as *in vitro* analyses of the interaction between specific collagens (or collagenous matrices) and $EIIIB^+$ FNs, should offer opportunities to assess the relevance of this segment to FN-collagen interactions.

The anti-EIIIB antibodies used in this study produced nonspecific N-glycanase-independent staining at the periphery of mouse RBCs, thereby creating the potential for deformable red cells in the microcirculation to generate "ring" and/or tubular staining patterns. Such patterns might be expected to be particularly evident against the "dark background" of densely cellular tissues, such as muscle and brain. Therefore, we have reported instances of N-glycanase-independent capillary-like staining patterns in such tissues with qualification (Table I). Nevertheless, authentic N-glycanase-independent staining of capillaries does appear to be present in the central nervous system. For example, in the choroid plexus anti-EIIIB antibodies produced distinct staining of capillary walls, while at the same time failing to produce lumenal staining (Figure 8). In contrast to capillaries, larger brain and meningeal blood vessels were stained by anti-EIIIB antibodies in an N-glycanase-dependent manner, suggesting that the walls of specific central nervous system vascular structures might be characterized by $EIIIB^+$ FNs in differing states of N-glycosylation.

The $B^+A^+V^+$ staining profile that we have observed in the vasculature and meninges of the adult brain is similar to the pattern produced by segment-specific antibodies in the brain of the mouse embryo (Peters and Hynes, 1996, this issue). Thus, despite reduced staining intensity in the adult (compare Table III, Peters and Hynes, 1996, with Table I, this study), we have not detected "wholesale" changes in the expression of these segments in the brains of adult versus embryonic mice. This agrees with the observation, made at the mRNA level, that the developmental downregulation in expression of the three spliced segments of FN is less pronounced in the brain as compared with the other organs in the rat (Pagani et al., 1991).

Tissue Distributi n of the EIIIA Segment

Among the isoform-specific anti-FN antibodies used in this study, those recognizing the EIIIA segment were observed to produce the most intense and widespread staining of blood vessel walls. Such staining was present in vessels of all sizes, ranging from the largest conducting artery (aorta) to capillaries. Particularly prominent staining was detected in structures conforming with non-fenestrated capillaries within striated muscles, in which anti-EIIIA antibodies showed substantially greater recognition of the microvasculature than of the endomysium, despite the presence of FN at both sites (Figures 1 and 5). Conversely, fenestrated capillary structures in the renal glomerulus (Figure 3), anterior pituitary, and adrenal failed to stain. However, in other cases (pancreas and thyroid), anti-EIIIA antibodies stained structures that appeared to conform with fenestrated vessels. Therefore, no clearcut segregation of EIIIA$^+$ FN expression according to the presence or absence of capillary fenestrations appears tenable. Also, as was observed in the mouse embryo, tissues lacking capillaries, including hyaline cartilage and Descemet's membrane, also lack EIIIA$^+$ FNs. This implies that A$^+$ isoforms might be generally incompatible with an avascular millieu (Peters and Hynes, 1996, this issue).

The observation that EIIIA$^+$ FNs are widely and preferentially expressed in the vasculature of the adult mouse suggests that they might constitute specific, and possibly necessary, components of the ECM within blood vessel walls. Several developmental and functional studies have previously implied such roles, both in the formation and maintenance of vasculature. For example, EIIIA$^+$ FNs are heavily distributed in the walls of blood vessels in the early embryo (ffrench-Constant and Hynes, 1989; Glukhova et al., 1990; Peters and Hynes, 1996, this issue), during which time FN is essential for proper development of the cardiovascular system (George et al., 1993; Georges-LaBouesse et al., 1996). Likewise, in adult animals EIIIA$^+$ FNs have been found to be overexpressed in the walls of blood vessels at sites of vascular injury and vessel wall hypertrophy (Glukhova et al., 1989; Samuel et al., 1991; Hosoi et al., 1993; Dubin et al., 1995). Conversely, intravascular release of soluble EIIIA$^+$ FNs has been shown to parallel real-time parameters of acute vascular injury and dysfunction, as might be expected of a component that is intimately linked to the structural integrity of the blood vessel wall (Peters et al., 1986; 1988).

The diffuse interstitial staining produced by anti-EIIIA antibodies in the adult lung is consistent with previous analyses of steady state levels of FN mRNA in rat and human tissues, in which substantial developmental regulation of the EIIIA segment was observed to occur in liver, but not the lung (Oyama et al, 1989; Pagani et al., 1991). Our findings are also consistent with the observation that FN extracted from adult rabbit lung is larger in molecular size than rabbit plasma FN (Peters et al., 1986), and is recognized by anti-EIIIA antibodies in Western blot analysis (J.H. Peters, unpublished). While it could be argued that these findings might simply reflect high-level expression of EIIIA$^+$ FNs in larger pulmonary blood vessels (see Figure 3, second row), such a restricted pattern of expression could not account for the observation that acute injury to the lower respiratory tract causes rapid release of soluble EIIIA$^+$ FN into the lung airspaces of the adult rabbit, without evidence of corresponding vascular release (Peters et al., 1988). The alveolar interstitium would appear to constitute the most likely source for such release, and this study provides evidence to support such an origin.

Tissue Distribution of the V Segment

In this first survey of the distribution of V$^+$ forms of FN in adult animal tissues, we have noted several instances of qualitative and/or quantitative differences between the staining patterns obtained with anti-V antibodies as compared with the other two segment-specific antibodies. For example, among the three antibodies, those recognizing the V region produced the most intense staining of a reticular network of fibers in lymph node and spleen. Anti-V antibodies also generally produced bright staining of epithelial

BMZs and their immediate subjacent connective tissues, whereas anti-EIIIA or anti-EIIIB antibodies typically produced little staining in these areas. Epithelia showing prominent V^+ BMZ and/or subepithelial staining included the cornea, skin, tongue (Figure 4), esophagus (Figure 1), trachea, stomach, intestine, gall bladder, vagina, uterus, oviduct, and urinary bladder. Thus, among the segment-specific antibodies, those specific for the V segment produce the most prominent BMZ staining in both the embryo (Peters and Hynes, 1996, this issue) and in the adult mouse.

Comparisons to Previous Studies

There have been several previous analyses of FN mRNA splicing patterns (ffrench-Constant et al., 1989; Oyama et al., 1989; Pagani et al., 1991; Magnuson et al., 1991; Jarnagin et al.; 1994) as well as protein isoform distribution (Vartio et al., 1987; Burton-Wurster and Lust, 1990; Laitinen et al., 1991; Glukhova et al., 1989; 1990; Carnemolla et al., 1989) in adult tissues. While our study is frequently consistent with these previous reports regarding the distribution of $EIIIA^+$ FNs (e.g., absent from cartilage and liver, present in kidney and brain), we have observed qualitatively different anti-EIIIA vascular staining patterns in comparison with those reported previously for human and rat aorta (Glukhova et al., 1989; 1990) as well as human skeletal muscle (Vartio et al., 1987). We have additionally noted a more diffuse pattern of staining for the EIIIA segment in the lung parenchyma than has been alluded to (Kuhn et al., 1989). Likewise, in the one previous immunohistochemical survey for $EIIIB^+$ isoforms in adult human tissues, only synovia, myometria, and some small ovarian interstitial blood vessels were reported to be positive (Carnemolla et al., 1989). Our observations are consistent with this latter report regarding synovium, myometrium, and ovarian blood vessels. However, we have additionally detected staining for the EIIIB segment in choroid plexus, meninges, esophagus, large intestine, urinary bladder, vagina, endometrium, oviducts, the walls of certain medium and small sized arteries, hyaline cartilage, and the eye.

The above-cited discrepancies could arise from a variety of factors, including species differences in FN expression, age differences between animals (we have analyzed relatively young mice), differences in antibody sensitivity, differences in epitope masking (potentially a greater problem with monoclonal antibodies), and epitope modulation on the basis of FN splicing (Carnemolla et al., 1992). With respect to the last point, the previous analyses of the tissue distribution of $EIIIB^+$ FNs relied upon a monoclonal antibody (BC-1) which recognizes an antigenic determinant(s) in the constantly expressed seventh type III repeat of FN. Although evidence suggests that the BC-1 epitope is revealed only in $EIIIB^+$ conformers of FN (Carnemolla et al., 1992), characterization of this antibody will remain incomplete until the effects of inclusion of the EIIIA and/or V segments upon its recognition of $EIIIB^+$ FNs is tested. On the other hand, given that inclusion of the EIIIB segment can affect the conformation of a constantly expressed region of FN, then it seems reasonable to suspect that such a change in splicing might also affect the exposure of epitopes located in another spliced region. If this were the case, then the inclusion status of one alternatively spliced segment of FN might potentially regulate the immunologic detection of a second spliced segment.

In summary, we have found divergent tissue staining patterns for the alternatively spliced EIIIB, EIIIA, and V segments in the adult mouse. We have also found substantially wider spatial patterns of distribution for these segments than previously documented. While the functional significance of these findings remains uncertain, the heterogeneous patterns observed in this study strengthen the contention that the three spliced segments of FN serve different functions, possibly involving interaction with distinct ligands, in various adult tissues and organs.

Acknowledgements

We would like to thank Jeffrey Barnes, Jonathan Said, Michael Fishbein, Irene Davos, Mario Fournier, John Young, Harry Vinters, Nathan Friedman, Alberto Marchevsky and Alda Vidrich for their gener-

ous discussions, advice, and/or technical assistance. J.H.P. was supported by a Program of Excellence grant from the National Heart Lung and Blood Institute (P01HL4184) and by a grant from the National Cancer Institute (P01CA17007), both to R.O.H., during the early phases of this study. J.H.P. was also supported in part by a grant from the Charles B. See Foundation. R.O.H. is an Investigator of the Howard Hughes Medical Institute.

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